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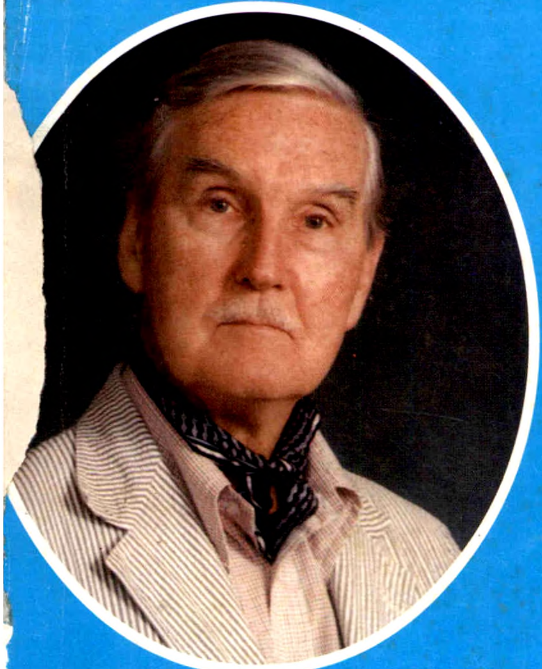

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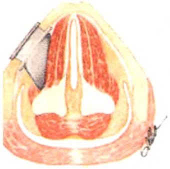

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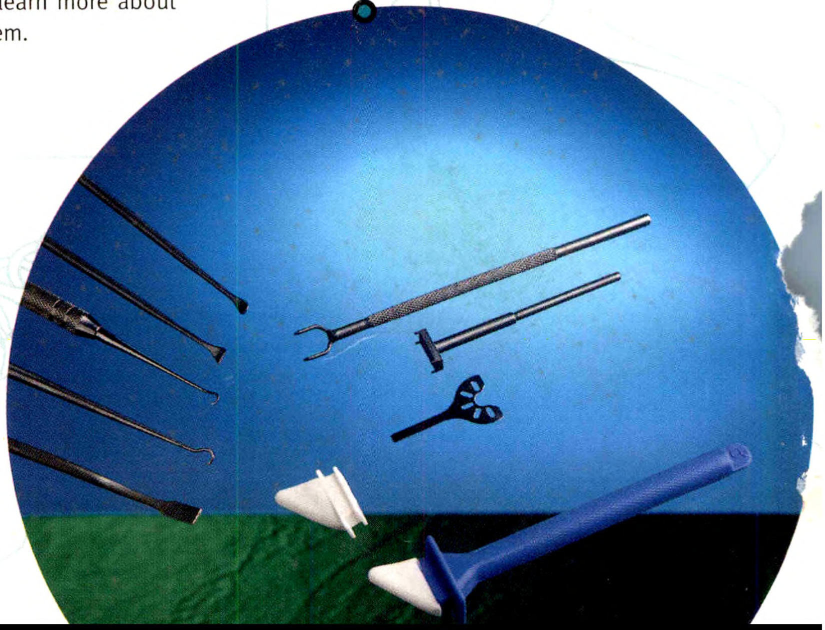

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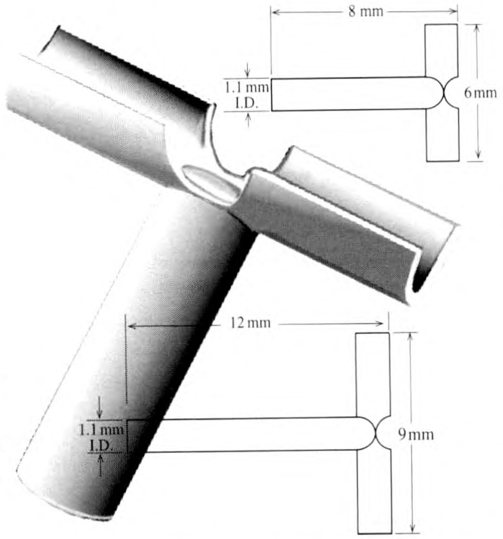

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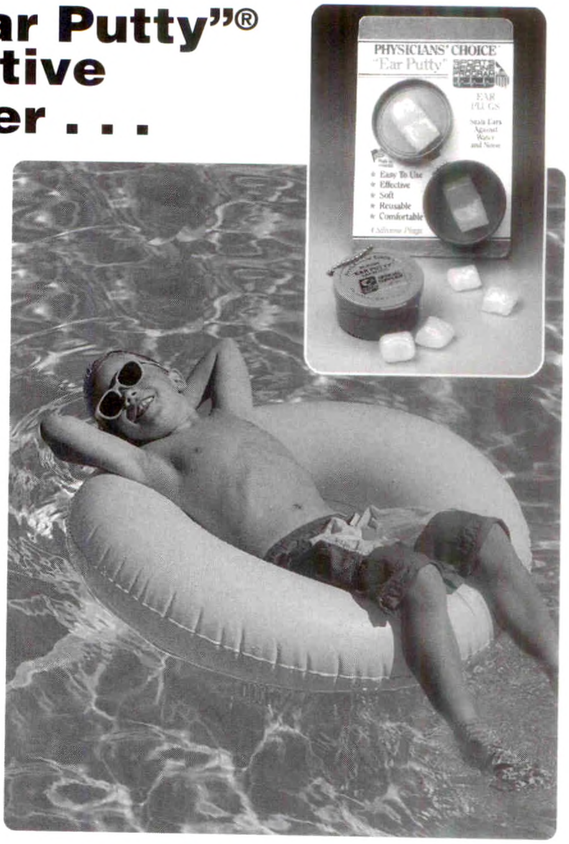

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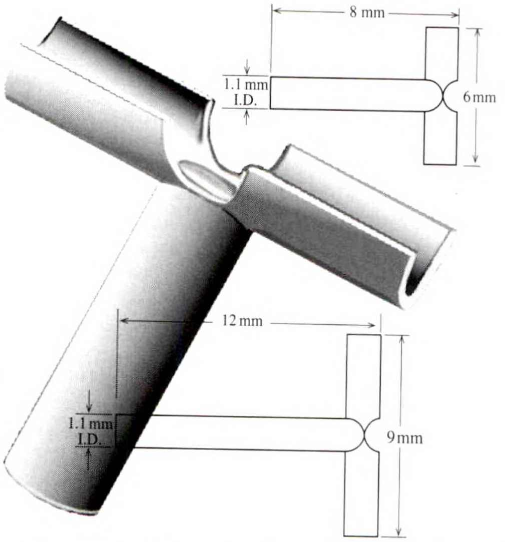

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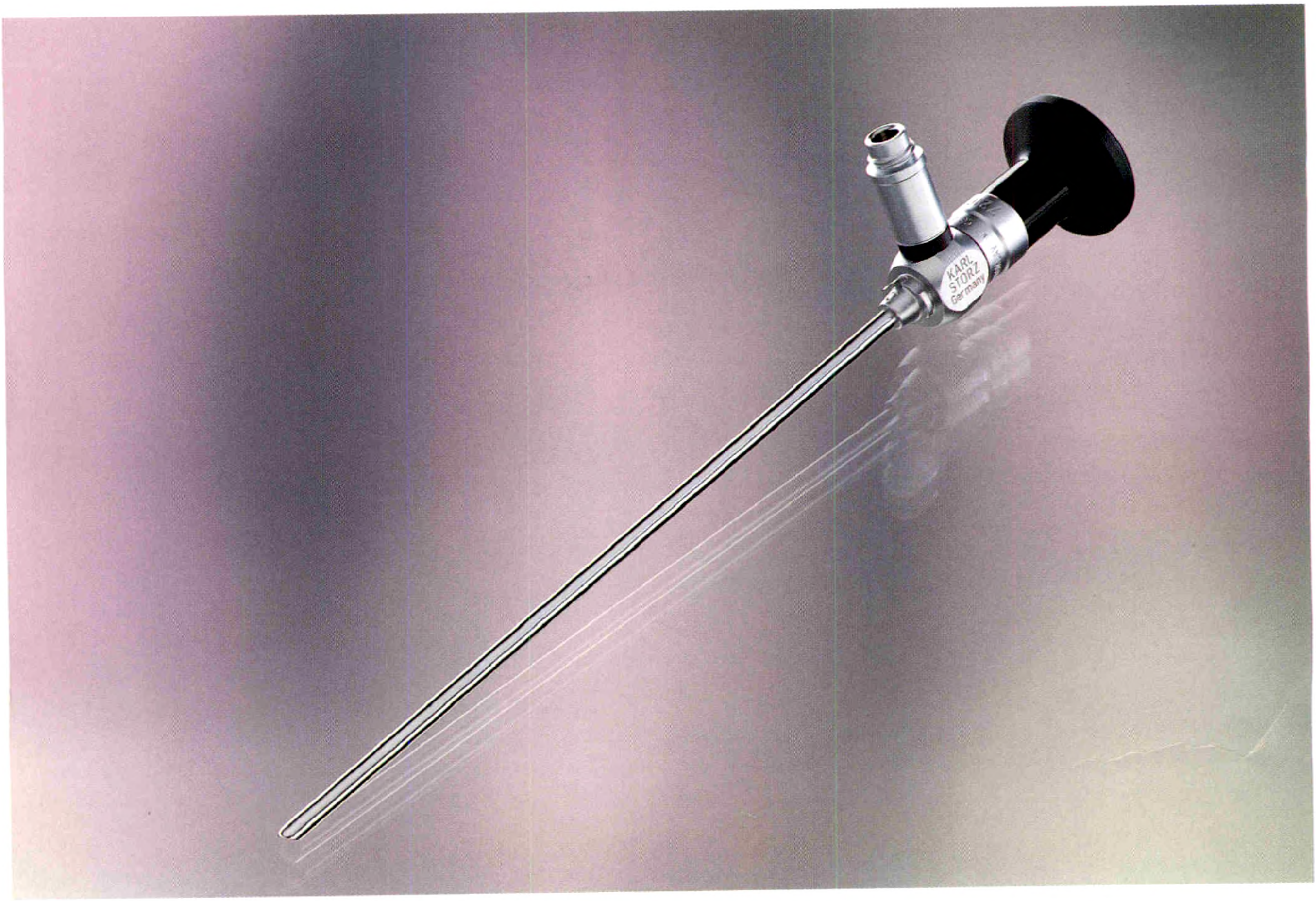

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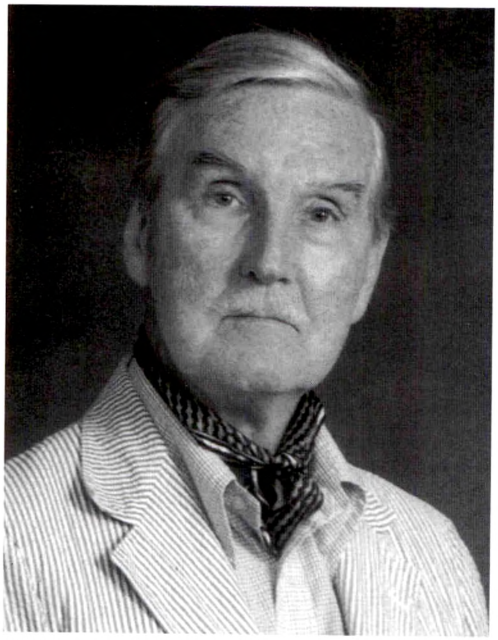
JOHN J. CONLEY, MD, 1912-1999
— IN CELEBRATION OF A LIFE —



A MEETING OF FELLOWS AND FRIENDS

There is no equal,
Indeed no mimic
Or facsimile in feeling,
To the flesh of the "Fellows"
And those gianted "Friends",
Who grew with me
In a peril of tension
That we might loose the truth,
And this threatened dream —
As real as the heart
Without sardonics of pain —
In a surgical sincerity
Beyond the dullness of work,
Laced with that discovery
That made us more
Than our hopes
By living one on one
In open intimacy,
Destined to forge this union
By the uniqueness of love
For your gift to my heart,
And my friendship for your life

— John Conley, MD

On the afternoon of November 19, 1999, just such a meeting as described in his poem took place in the auditorium of the New York Academy of Medicine.

It was a meeting, however, that was without the physical presence of our great mentor, although his spirit permeated the hall and the hearts of all the "fellows and friends" that were present that day. This was his memorial. Indeed, John anticipated such an event, for he had penned in his latter years, among notes on his wish for a plain casket and rapid interment, that he had "no objection to a small sensitive gathering in a few days or a few weeks after my burial, as Monika would desire." This could not be, however — because he was held in such high degree of admiration and respect that everyone who could, came. And the small group John envisioned grew by word of mouth to almost 200.

Below are recorded the eulogies of ten of his fellows and friends, which taken together and severally, comprise a paean of praise of this Renaissance man who raised head and neck surgery to an art form, and gave to us unstintingly as a teacher with virtually no equal.

BRIAN F. MCCABE, MD

Members of the Conley family, ladies and gentlemen, fellows and friends. This afternoon we assemble to celebrate the life of Dr John Conley and also to mourn the loss of a husband, father, friend, most distinguished colleague, mentor, and role model without peer. John Conley was a man of strong personal convictions, forthright and honest in his association with colleagues, and endowed with an equanimity of spirit endearing to all with whom he associated. In his personal relationships he was always gracious, always courteous, and always considerate. And despite having himself achieved the heights of professional attainment, he could always find time for those of lesser stature and he fully enjoyed his role as a mentor and teacher. He was a physician in the finest tradition of the profession: dedicated, skilled, creative, and compassionate. The heritage of his chosen specialty was immeasurably enriched by his accomplishments, and few individuals have had a greater impact on otolaryngology–head and neck surgery than John Conley. During times of conflict with other disciplines, John, in his inimitable way, was a pillar of strength, providing legitimacy and credibility for his chosen specialty — one of his most enduring legacies.

John once described his fascination with medicine and surgery as a "love affair." As one of his fellows, I had the good fortune to experience firsthand his passion, energy, and commitment. His patients, students, and colleagues were all beneficiaries of the romance.

John's passion was never more evident than in the operating room, where his artistic inclination and love for innovation offered moments to treasure. He was in his element, and those privileged to have observed him perform surgery can testify to the electricity and excitement he created as the master surgical technician. His manual dexterity was deliberate, delicate, and precise when indicated, and again, where called for, in the complicated high-risk situation, rapid, utilitarian, and courageous.

John's love of teaching all aspects of head and neck surgery and his vast knowledge and experience were a motivating force for all of us who worked with him. We were privileged to witness firsthand his interaction with his patients, and were impressed by his humanity and compassion. Most of his gravely ill patients were obviously terrified by their disease. He comforted and encouraged them in a manner that was special to behold. They in turn loved and revered him and continued to inquire about him long after he stepped back from surgery.

Sir William Osler, in "Aequanimitas," stated of one of his colleagues, that "to have known him was, in the deepest and truest sense of the phrase, a liberal education —

Whatever way my days decline,
I felt and feel, tho' left alone,
His being working in mine own;
The footsteps of his life in mine."

The same can surely be said of John Conley by those privileged to have known him, not only as physician, teacher, and ethicist, but also as a talented musician, painter, and poet. He was in every sense a Renaissance man and, indeed, a "liberal education."

And so, John, God speed you on your journey to the peace and quiet of eternal rest, and know we will greatly miss your energy, gentle spirit, and wise and friendly counsel.

STANLEY M. BLAUGRUND, MD

Bis vivit qui bene vivit. (He lives twice who lives well.)

The young invariably view the past as of little consequence, and it is only with the passing years that the contributions of those that have gone before become truly appreciated.

I believe I first met John at a Triological Meeting in San Francisco in 1962, just after I had become a professor of ENT in London. I was a new boy, unproven, and at 37 a tyro in the emerging field of head and neck surgery. Oscar Wilde said a man should be judged by the effect he has on his friends. Well, John accepted me as a friend, providing an example to be followed for the whole of my professional and ethical life. We met intermittently, usually in different parts of the world, where we continued discussing topics left in limbo at past conversations. Despite the age and cultural differences, for John's artistic sensibilities were far different from my more pragmatic background, we shared far more beliefs than many could believe. Few fathomed the deep trust that lay between us, when listening to the lighthearted banter that was such a feature of our "professional" conflicts. The British reserve such verbal banter for those they trust and revere, and these were certainly my feelings for this predominant figure in world head and neck surgery. After I retired, we continued to exchange letters, and gradually I began to appreciate the deep feelings that lay beneath the often incomprehensible prose of his poetry. John used the English language like no other, providing me and so many others with constant delight. It was obvious to his friends that Monika brought him great happiness, reflected in his poetry, countenance, and well-being. It seems somewhat unjust that his end should be associated so intimately with the very disease that he had overcome so successfully for much of his life. Thank you, John...*Aeternum vale*...Farewell for ever.

SIR DONALD F. N. HARRISON

Although he looked and sounded like an English nobleman, Dr John Conley was born in Carnegie, Pennsylvania, a small steel mill town just outside of Pittsburgh. He graduated from the University of Pittsburgh and later its school of medicine. He interned at Mercy Hospital in Pittsburgh. During that year, John was very popular with the nuns who ran the hospital. They suggested that he take a residency in cardiology and come back to Mercy as their cardiologist. He went to Kings County Hospital in Brooklyn, a very busy city hospital with a huge patient population. Shortly after he began his training, he had an arrhythmia diagnosed as paroxysmal atrial tachycardia. Little was known about this benign condition at that time. John was told that cardiology was too stressful and that he should go into an easier, less-stressful field with better working hours, like ENT. He did an otolaryngology residency at Kings County Hospital. This was followed by four years of military service during World War II, which included experience in otolaryngology and plastic and reconstructive and maxillofacial surgery in the United States Army Medical Corps, both in this country and in the South Pacific theater. This exposure to the reconstruction of war wounds would prove invaluable to him later on in applying these principles to reconstruction following ablative head and neck surgery.

John returned to New York City after the war. He became an assistant and then an associate of Dr George T. Pack, a technically superb general oncologic surgeon at Memorial Hospital who taught John major ablative surgery of the head and neck. They worked day and night catching up with the backlog of surgery that was neglected during the World War II years. The combination of his training in otolaryngology, the exposure to ablative surgery, and the World War II experience in reconstructive surgery set the stage for Dr Conley to evolve his unique approach to head and neck surgery.

Ironically, despite the admonition of the cardiologists about hard work, John did a prodigious amount of major head and neck reconstructive surgery. This proved to be more than ample to provide training to many fellows like myself. His commitment to education is further attested to by the position he held many years as Clinical Professor of Otolaryngology at the College of Physicians and Surgeons at Columbia University. He loved his appointment at Columbia and particularly his involvement in teaching the residents. His long friendship with Dr Daniel Baker, Jr, chairman of his department, was an aspect of Columbia he also cherished. I think it was quite fitting and very satisfying to John that the John Conley Lectureship was established two years ago at Columbia by Dr Lanny Garth Close, chairman of the department. I had the honor of being the second Conley Lecturer in June.

His vast surgical experience, together with active research interests, led to the authorship of almost 300 contributions to the scientific literature, and eight books. As a result of his productivity and rhetorical eloquence, he was very much in demand as a speaker in this country and abroad. He gave many prestigious eponymous lectures in our field and received many awards for his work, including the Philip H. Hench Award as the Distinguished Alumnus of the University of Pittsburgh School of Medicine, and the DeRoaldes and Newcomb Awards of the American Laryngological Association.

John's contributions to the scientific literature, his many technical innovations, and his surgical experience placed him in the position to receive many honors and important leadership positions in our field, such as President of the American Academy of Otolaryngology and Ophthalmology, a member of the Board of Governors of the American College of Surgeons, a founding member of the Society of Head and Neck Surgeons, and a founding member and first President of the American Society for Head and Neck Surgery. During those years, Dr Conley used, to the great benefit of us all, his wisdom and diplomacy in carrying out these high-level responsibilities.

John's work throughout the years was very successful, because although he was a man with a very complex character working in a very complex field, he developed a philosophy and techniques of great simplicity characterized by the centrality of a theme that could be adapted by those like myself who worked with him closely as fellows. His contributions to head and neck surgery are characterized by a wonderful coherence, and his overall presence had the additional luster of his cultural achievements.

Some years ago I had the opportunity of attending a dinner at which Dr Herbert Rosenbaum was the guest speaker. Dr Rosenbaum wrote a book entitled *A Taste of My Own Medicine*, which was transformed into a movie called *The Doctor*, which starred William Hurt. This was the story of a physician with cancer of the larynx who, for the first time in his life, instead of being a physician in control of his illness, became a patient with no control over the illness. The doctor was treated with radiation therapy. He retained his voice, was cured of his disease, and finished out his medical practice. Dr Rosenbaum was pleased with the technological advances that resulted in the happy outcome in his case. Dr Rosenbaum's major theme, however, was not that of technology but rather ethics, professionalism, humanism, and doctor-patient relationships. He talked a great deal about how doctors are viewed by their patients and the public as being arrogant and living in their own world and being detached and noncommunicative with patients.

Among the great legacies that John Conley left to all of us whose lives he touched is not only the benefit of his keen intellect and his ability to perform and teach surgical technique, but his philosophy that the physician should comport himself ethically and with dignity and humility and should never lose the opportunity to be seen by his patient as an individual who is interested in his or her problem and is willing to devote the time and power of concentration to listen to what his patients have to say and to help in this way to bring the doctor-patient relationship to the very highest level. John Conley was the ultimate example of professionalism and was a master at making all of his patients feel important, well taken care of, and reassured. It didn't matter whether the patient was a wealthy businessman, Secretary General of the United Nations, or a blue-collar worker. He knew that these patients were frightened out of their minds by their disease and that neither their money nor their power could help.

I've been around long enough to know that surgical techniques and technology come and go, but the theme that always dominates patient management is the ethical and spiritual relationship between the doctor and his patient.

John Conley was a man for all seasons and was an accomplished musician, artist, and poet. He was also an extraordinarily sensitive person, which influenced his behavior and engendered trust and confidence, which helped the patient to accept the often debilitating surgery that was necessary to save his or her life. In the office and on rounds, I always marveled at how John dealt with patients and their families with style, honesty, and forbearance. The words he spoke to them were some of his best poetry.

EUGENE N. MYERS, MD

It is with consternation and a feeling of deep grief that we learn about the death of our great surgical and human idol, Professor Dr John J. Conley.

By an inconceivable fate, a primary tumor at the skull base, involving the facial nerve, and another malignant growth in the parotid region put an end to the life of our great John Conley. Both tumors, with their perilous nature and their life-threatening quality, were a constant challenge and a matter of continued interest to the greatest surgeon in our specialty.

It was John Conley who devised the big, comprehensive block resections for these dangerous tumors and thus also became the pioneer of modern extratemporal facial nerve surgery. He worked out the surgical concept of the principal integration of far-reaching block resections in head and neck tumors with immediate reconstructive surgery, and in this way created the field of modern head and neck surgery. It is a terrible tragedy that in the end, the great master himself fell victim to a deadly combination of such tumors, tumors he had combated with all his ingenious skill most of his life. With his surgical concept, John Conley was able to restore human dignity to innumerable cancer patients.

It cannot be valued too highly that after World War II, with its devastating results for the international community, John Conley belonged to those representatives of our field in the United States who were most actively involved in bridging the wide gaps that had opened in professional as well as human relationships. It was mainly due to his vision that German otolaryngology widened its scope to include head and neck surgery, and in 1997, in recognition of his outstanding contributions, the medical faculty of the University of Goettingen took pride in bestowing an honorary doctorate on Professor Dr John J. Conley.

PROF ADOLF MIEHLKE

Where do you start when you want to recognize and pay respect to a great physician, a great teacher, a great inspiration to us all — and a great friend? A philosopher, medical ethicist, orator, and patron of the arts? Since John's departure, many eloquent things have been said and written, and more has and will be said today — more eloquent, to say the least, than I would even attempt regarding his accomplishments and his creative influence on the broad realm of our specialty. My remarks will be of a more personal nature, to emphasize John's character, not just as it related to me.

Certainly, as you may be able to tell from my accent, I'm not from New York. I did not have formal training from Dr Conley, yet he taught me so much, was a mentor for my entire professional career, and made me feel like I was a truly close friend. John had that special ability. He made many of you feel the same way, I'm sure. Those of you who did train with him received an extraordinary privilege.

From one meeting to the next, both personal and professional, he would remember what you were doing professionally, "for the good of the cause," and personally would include the names of your children, especially if there were any problem areas. His lovely and talented wife, Monika, had the same knack, so as to endear both of them to you.

Whatever laurels we may earn in our lives, we all like to make a good impression on our children. After the Academy of Otolaryngology–Head and Neck Surgery meeting ten years ago, when I presided as president, John courteously stayed down in front of the stage to talk with me, and in the presence of my family, said, "Richard, that was the best meeting ever," and with one of his dramatic gestures, exclaimed, "That program had your stamp all over it." What a guy!

Later, he expressed the same sentiments in his own writing, when I received my next copy of *Vocal Painting*, his book of poems — so ethereal and so deep that only sparingly did I understand all of them, except the one titled simply "Monika." We all like to get compliments from such a distinguished person. John was so generous with these.

At that meeting I had his friend and compatriot, Edmund Pellegrino, as the John Conley Scholar. John was delighted I had chosen a medical ethicist. At the same time, I projected on the screen one of those immaculate, dapper pictures of John and said, "We would all like to have John Conley as our guest of honor at every meeting." We all thought so much of him. Stan Blaugrund, who has been so helpful during these trying times, had him as his guest of honor when he was vice president of the Eastern Section of the Triological Society, the last meeting I attended in New York honoring John. At the time of that meeting, John established the John Conley Foundation for Ethics and Philosophy in Medicine. This has expanded into an ethics essay contest for medical students. Some fade away, but John Conley will be remembered for generations.

We both watched each other operate in this country and abroad. He was politely complimentary, and I was in awe. Such a delicate surgical touch — such respect for the tissues.

I have known John for nearly half a century and was always in awe. I would like to think I actually influenced "the great one" toward joining and giving his stature to the American Academy of Facial Plastic and Reconstructive Surgery. We spent two hours talking about this in a quiet corner of the lodge at Lake Placid at a Triological meeting years ago. I was pleased and thrilled he gave me this much time. Of course, he did join, was elected the third president, and then gave the first course on face-lifts at the then American Academy of Ophthalmology and Otolaryngology meeting.

He immediately preceded me as president of the plastic surgery academy — what an act to follow. He and I were on the first plastic surgery faculties at the University of Wurzburg with Professor Wüllstein and in Zagreb with Professor Padovan — an opening for the subspecialty in Europe.

I could talk for five hours, not five minutes, about our relationship from the beginnings of the head and neck society and the facial plastic academy, promoting these disciplines for the otolaryngologist, the battles that were fought and the wars that were won, most under John's leadership and guidance — extending to playing golf with him when he visited in Tampa. I didn't even know he played. He accepted my invitation and shot a most respectable game — unflappable.

One last story. In Chicago, at a board of directors meeting of the facial plastic academy, I had invited my former chief, Dr Dean Lierle from Iowa, as a special guest to talk about our future, training, and interdisciplinary considerations. He had some controversial things to say and asked that his remarks be "off the record." Lierle's gruff manner, in contrast to Dr Conley's, was graphic. Dr Conley followed and, as only he was able to do, gave one of the best extemporaneous talks I have ever heard — in the course of which he gestured with his elegant hands and stated dramatically, "This is our region." Soon after that, we all started talking and writing about the "regional surgeon." Most unfortunately, this historic speech of John's was not recorded. The great orator! What a command of the language.

His daughters would not remember, but I had the special privilege of having a private dinner with them in New Orleans when they were young and neither had married.

Where do you stop talking about the greatest head and neck reconstructive surgeon of this century? I think I should stop now.

Monika, John, Mary Downe, Anna, and Mary Conley, we all loved him for all that he did for us, as a unique, realistically spiritual, and unparalleled human being.

RICHARD T. FARRIOR, MD

It is indeed a privilege and an honor for me to be here with all of you, reverencing the memory of Dr John Conley. I join everyone else in expressing my condolences to his wife, Monika, and to his family, and I add my own prayers for Dr Conley's eternal rest and happiness.

I think that aside from his patients and his family, I am perhaps the person who has benefited most from Dr Conley. I am the first Chair of the John J. Conley Department of Ethics at Saint Vincents Hospital and Director of the Bioethics Institute at New York Medical College. In that light I have benefited greatly from Dr Conley's continuing generosity, his energy, and most of all, his vision. Perhaps in no small measure, the Sisters of Charity, with whom Dr Conley worked at Saint Vincents, helped to nurture that vision. It is well known that Dr Conley was a man of many talents: an excellent surgeon and innovator, a musician, an artist, and a poet. But, as others have also noted, he was concerned above all with ethics and professionalism in medicine.

In preparing my remarks, I actually did a little research and went through some of Dr Conley's papers and found that his sense of ethics pervaded much of his writing, going back into the 1970s.[1-8] The testimony given by others here corroborates the impression one obtains from the literature that this sense of ethics permeated all aspects of his career. For instance, it affected his practice, as evidenced by a paper entitled "Have I Performed the Right Operation?"[5] It certainly affected his concern for his patients as well, as evidenced by another paper entitled "The Meaning of Life-Threatening Disease in the Area of the Head and Neck."[4] And it affected his teaching, as evidenced by a paper entitled "Is Medical Education Meeting the Needs of Today's Physicians?"[6]

If one reads these papers carefully, one will also learn of his wit. One of his last papers, written in 1997, is an editorial arguing against assisted suicide.[8] It ends with this simple wry statement: "I see no great reason to disagree with Hippocrates in this respect." One can almost see the twinkle in his eye saying that.

The expansiveness of his ethical vision led him to found the John Conley Foundation for Ethics and Philosophy in Medicine. The title of this foundation is important. For Dr Conley, it wasn't just *ethics* in medicine. It was ethics *and philosophy* in medicine. He had a way of getting to the root of everything. This was certainly true of his approach to tumors of the head and neck. It was true of his way of connecting bench to bedside in terms of his surgical practice. It was only logical that it should also be true of his approach to ethical problems. The answers had to be founded upon the basic science of philosophy.

The John Conley Foundation, as many of you know, sponsors lectures at various hospitals, not just at Saint Vincents. The Foundation also generously endows a medical student ethics essay contest, and the winners are published in *JAMA* every year.[9] And, of course, the Foundation made a generous gift to found the John J. Conley Department of Ethics at Saint Vincents Hospital and Medical Center, a department that I hope will continue to get to the root of things: to make that connection between philosophy and ethics, between the bench and the bedside, between theory and practice.

My personal association with Dr Conley, compared with that of many of those gathered here, was really very brief. I met him only a few times, but he touched me as he touched many of you. And a few months before his death, although he was very ill, it was a wonderful privilege for me to see him come to Saint Vincents for a little open house for the John J. Conley Department of Ethics. He was able to see his name up on the door as part of the heritage that he leaves behind for the rest of us. He had the vision to know how critical ethics is to the practice of medicine and how it could only be sustained by the kind of generosity that he was able to give it. He had the vision to help found a *department* of ethics — not just a hospital ethics committee, but an actual department of ethics. This is a department committed, as he was, to patient care, teaching, and research, now in the relatively new field of medical ethics.

I want to close by quoting from one of his papers. In this paper, Dr Conley actually rewrote the Hippocratic oath into a set of commandments for all of us.[7] I think it would behoove us to pay attention to his words:

1. Be humble and proud you are a doctor.
2. Be grateful to your teachers.
3. Honor your colleagues and your patients with natural and honest respect.
4. Defend the rights and dignity of the patient.
5. Accept and help all patients, without exclusion, to the best of your ability.
6. Do not perform unnecessary surgery.
7. Inform the patient and his family about the disease process and the possible complications of treatment. Answer their questions, and recognize options and alternatives.
8. Do not give guarantees, but tell the truth as you see it.
9. Never hesitate to advise a second opinion, to seek help, or refer a patient to another doctor.
10. Be fair in your charges, and be generous to those who cannot pay. The professionality of medicine should be higher than business. Recognize the essentiality of business on all aspects of medicine and use it to improve medical elegance

and service.

11. Respect confidentiality.
12. Appreciate the complexities of illness, the hazards of treatment, and the sanctity of life.
13. Recognize that not all problems can be solved, and that there is often an association with religion, politics, ethics, and society.

We would all do well if we would continue to listen to Dr Conley.

DANIEL P. SULMASY, OFM, MD, PHD

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A legend has passed away, and we salute his journey on this planet, for it has touched so many, and in so many ways.

I met Dr Conley in 1967 at St Vincent's Hospital, where I was fulfilling my general surgery obligations. For the first couple of years, I was living under a pseudonym imposed on me by Dr Conley, because he thought I was related to the 18th-century Czech composer whom he very much admired and who had a similar name. But it was Dr Conley's intimate knowledge of the composer's music that provided for me the first connection with Dr Conley, in addition to the daily complexities of surgery.

To work with Dr Conley was a unique and life-changing experience for all the residents and fellows who were fortunate to have that opportunity. It was not easy for us young residents to fulfill all of what we thought were Dr Conley's expectations, real or imagined, as he was to us bigger than life. The aura of his personality always preceded the start of our rotations, as did the daily dose of apotheosis generated by word of mouth in the hospital cafeteria by all those young physicians who "lived to tell the story," as well as by the frequently overheard staccato phrases emanating from Dr Conley's operating theater: "Help the doctor," "Attaboy," and mostly "Suck, suck, suck!"

In the OR, Dr Conley was a commanding figure in his noble struggle with the demon we call cancer. His form of exorcism was a radical surgical ablation, of which he had many variations with descriptive terms only Dr Conley could coin: "the standard," "the mini," "the maxi," and "the all-American technique" — often equivalent in design and execution to the medieval crusaders, in terms of the voracity, the determination, and the noble goals. It was not, however, only a "burn and destroy mission" (practiced by so many at that time), as he always wanted to return the patient to a state of "reconstructive convalescence," often ending with return not only of life but function as well. Dr Conley's fingers were like the dancing Wu-Li masters, leaving a legacy of a quantum leap in head and neck surgery.

Dr Conley did have a human side, blossoming with jubilation when Monika entered his life. It was like standing in the light spring drizzle with bright sunshine coming through forming a rainbow when he talked about her, sitting with us on a hospital stretcher waiting for his next case.

And for those of us who believe that this is not the end — and for those who believe, no proof is necessary, and for those who don't believe, no proof is possible — let's immerse ourselves in T. S. Eliot's words: "And the end of all our exploring will be to arrive where we started and know the place for the first time." Godspeed, Dr Conley.

IVO P. JANECKA, MD

John Conley, MD, will be remembered as an extraordinary man, with exceptional vision, courage, and intellect. Of his many outstanding traits, I would like to share three that were particularly noteworthy in his roles as a physician and mentor.

The first was his unlimited intellectual curiosity. He had an extraordinary breadth of interests and a phenomenal ability to absorb new knowledge. His interests extended far beyond surgery and medicine. As is well known, he was a fine poet, musician, and painter. He played flute, accompanied on occasion by his wife Monika, who plays the harp. He published many volumes of poetry, deep in meaning and eloquent in thought and form. He painted in many styles, often reflecting the influence of modern abstract artists, but with his own stylistic edge and sense of humor.

In 1987, I had the honor of being Dr Conley's penultimate fellow. In this role I witnessed firsthand his curiosity and seemingly infinite capacity to absorb ideas and to synthesize them into new perceptions. This capability was all the more phenomenal, in that, at the time of my fellowship, he was already in his mid-seventies. Yet his analytical prowess and intellectual curiosity remained vibrant. At a stage of life when many men fall back on platitudes and stale truisms, he had an exceptional ability to remain open to new and sometimes unfashionable ideas.

One morning during my time with Dr Conley, a limousine pulled up in front of his Central Park West office in New York City, and a woman and man entered. The woman was examined and was found to have a mass of the parotid gland, which would need an operation. She had three requests regarding her surgery. First, she insisted that her identity not be revealed. She also requested that crystals be allowed in her hospital room after the surgery to promote healing. Finally, she requested that a faith healer be present postoperatively to speed her recovery. Dr Conley had treated many celebrity patients, and was prepared to honor her request for anonymity. As regards the healing crystals and healing hands, he made the remark, "We do not know everything about the human body and about healing," and was quite accepting of her nontraditional approach. His reflection on the limitations of the medical model was at the time bold, for it was made in an era that predated "integrated" or "complementary" care. This openness to unusual concepts was not unusual for Dr Conley, and I saw on many occasions patients relieved to find a surgeon who could accept the unconventional and meld it with traditional medical care.

Immediately after the surgery, the anonymity was broken when the woman's press secretary announced that she, a famous musician, had successfully undergone surgery for a benign tumor. Large quartz crystals and a faith healer took their place in her hospital room. Her partner, a manager for professional wrestlers, expressed his gratitude by providing two tickets for ringside seats at Madison Square Garden for professional wrestling. So, I had the pleasure of accompanying Dr Conley to see the World Wrestling Federation, "up-close and personal." Hulk Hogan, in his prime, was throwing bodies in and out of the ring. Dr Conley rather liked the theater of it all, and commented on the gritty reality of the competition and its parallels in life. Despite the fact that his personal style was completely dissimilar to that of the characters in the performance, he retained the capacity to accept a new experience, to extract its essential qualities, and express how it enhanced his appreciation for the richness and experience of life.

This event particularly stands out in my memory, for I am certain Dr Conley was one of the few people ever to have witnessed professional wrestling, ringside at Madison Square Garden, with a small, round, metal Museum of Modern Art attendance pin neatly folded over his herringbone lapel. He truly was a Renaissance man.

The second characteristic of Dr Conley that I found outstanding was his sense of leadership. All of us in otolaryngology are in debt to Dr Conley for his unfailing dedication to the specialty of otolaryngology–head and neck surgery in times of change and challenge. He was articulate, strong, principled, and willing to assert his opinion to further the cause of the specialty and to ensure that patients received the finest in care. We owe a great deal to his vision and courage.

Finally, John Conley was a master of his craft. He had high expectations of his students, residents, and fellows, and valued thoroughness, attention to detail, and personal responsibility. He expected total focus on each surgical operation, with reflection about the case before, during, and after the procedure. Every patient and every operation was an opportunity to learn or teach. There were no routine procedures.

Early in his career, Dr Conley had the opportunity of serving in the United States Army at Fort Dix, New Jersey. He once recounted that he was the attending physician for a 300-bed surgical ward for wounded soldiers returning from the European front. He was responsible for general surgery patients, plastic surgery patients, and also neurosurgery patients. His awareness of the complementary surgical skills and techniques used to treat these three types of patients led to his developing new surgical approaches for head and neck surgery. This experience was the genesis for combining ablative surgery and reconstructive surgery for head and neck cancer patients, which was to become his hallmark. Not only was he creative, but he shared his insights with colleagues and students with energy and excitement, challenging them to take on difficult questions about disease, healing, people as patients, and medicine as a profession.

Dr Conley was asked some years ago, were he to change anything about his career, what would he do differently? He responded that he would have learned more about business. I do not know if this is because this is the one field that he felt he never conquered, or if he could see that medicine was increasingly becoming engulfed in mega-mergers and profit margins. I feel fortunate to have had the privilege of studying with a mentor who was a physician first. His personal and professional ethics and understanding of patients clearly set a standard for the entire profession of medicine.

Thank you, Monika, for your loving attention to John, and for the invitation to speak at this service today. It is an honor to participate in this memorial to a great and gentle man.

CARL A. PATOW, MD, MPH

I am grateful I can say that John was my friend. How it happened that he took an interest in an obscure practitioner tucked away in the puckerbrush of Maine, I never could fathom; but over the years he demonstrated his friendship many times, in many ways. Perhaps it was his own rural heritage that influenced him.

In matters related to eternal life, I am no expert. I would like to believe that in our afterlife we would be comfortably located on a fleecy cloud, enjoying the company of predeceased family and friends, renewing old acquaintances, and enjoying our favorite pursuits in peace and harmony for eternity. Can't you imagine John ensconced on a fluffy cloud, surrounded by family, friends, and colleagues, his painting palette and writing materials nearby, with his recorder tucked securely under his arm, serenely strumming his harp? I hope this will come to pass, although I am uncertain about the prospect, but I am certain that there is immortality for each of us on a different plane here on earth. As ripples spread from a pebble tossed into the millpond, our deeds, while alive, will spread and influence the lives of countless others, whose lives will continue to spread our message and influence as-yet-unborn generations.

John's impact on medicine and on his associates came from the fact that he was the caring physician and consummate surgeon in the best sense of the word, and what is even more important, he was ever a high-minded gentleman. It was his desire to see humanism an integral part of medicine, a role he lived as an example, and a cause to which he contributed largely.

He was never too busy to teach. From the medical student to the skilled surgeon, at all opportunities, he generously imparted his knowledge to his colleagues, seasoned with his empathetic approach to both patient and family. He provided a month's preceptorship for a young man, early in his surgical residency, to acquaint him with the proper way to manage patients, their families, and their problems. The time spent with John influences his practice today. To quote him (Donald C. Lanza), "It was a fabulous experience."

John was a devotee of the arts, especially of music, and fostered their development in young, talented people by generous support through scholarships and fellowships. He was himself a painter, musician, and poet of great ability and directed his philanthropy to these and to other diverse fields of interest to him.

John was a classy person, always dignified, reserved, and appropriate in dress, demeanor, and act. On a trip to China, beset with some inconveniences, he tolerated all of the problems and maintained his usual good humor throughout the entire trip.

His legacy will be found distributed among the many lives he influenced — family, friends, patients, colleagues, and students — and like those ripples spreading from the stone cast into the water, his influence will be felt for eons in the future, even long after his name is no longer readily recalled. This is John's certain immortality and will constitute a most satisfying and enduring earthly memorial.

I consider myself fortunate to have been numbered among those who could call him friend, and to be one of the ripples within his sphere of influence.

LORING W. PRATT, MD

A giant of creativity — separated from the ordinary by an aura of genius. Awe-inspirational, an air of unapproachability about him — yet extraordinarily accessible to those who dared to inquire.

A savant, held by all as one possessed of intuitive judgment...unencumbered by the traditional dynamics of medical politics...free to personally interact with issues and not consequences.

His needs transcended the sequelae of commercial enterprise. Quality control was measured by an ethereal musical note or a penetrating word...not by the cacophony of electronic life.

Support of the dreams of the artistically endowed was obligatory, a necessity to ensure cultural perpetuity. A self-imposed commitment that enabled an elevation of our own value systems.

An explorer of sorts, pushing the unknown of surgical boundaries...discovering, then charting, the new terrain...once confirmed, casting this sphere of accomplishment to his disciples. Once an outlier in the world of surgical tradition...he assumed authorship of a new awareness and higher level.

A sartorial nonconformer; stylish to himself, blurring the traditional requisites of coordinated color. Occasionally the hairgyle replaced the argyle.

Loyalty is defined by those in receipt of his surgical expertise...as recognition of this surgical purity and directness of the spoken word...honestly crafted, an enlightened relationship free from the impediment of tactical thought.

Ethics reigned as a supreme interest...a calling to influence human behavior in the most positive way...especially as it relates to the sick and dying. All in his chosen discipline, either knowingly or unknowingly, have been positively affected toward this end.

His essence was multifaceted and many-dimensional, shaped by his extraordinary natural talents, molded by his all-encompassing quest to understand.

A giant of creativity — his genius refracted by the prism of life...such that all (family, patients, colleagues, artists, and musicians) are able to see somewhat more clearly.

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HARVESTING HUMAN HAIR CELLS

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The sensory hair cells of the inner ear are responsible for converting balance and hearing stimuli into electrical signals. Until recently, all previous studies of hair cell physiology had been performed on tissue obtained from non-mammals and rodents. In primates, hair cells are difficult to access, because they rest within the densest structure of the body, the otic capsule of the temporal bone. In this report, we describe a technique that we have used in physiological studies to harvest living human hair cells. We collected vestibular and cochlear tissue specimens from adult humans undergoing translabyrinthine and transotic surgical approaches for resection of lateral skull base tumors. Viable hair cells were identified and visualized with light microscopy. The ability to study normal hair cells from humans may further the study of normal and pathological human sensation, hair cell regeneration, and genetic causes of balance and hearing disorders.

KEY WORDS — acoustic neuroma, balance, cochlea, electromotility, hearing, ionic currents, otology, primate, vestibular organs, vestibular schwannoma.

INTRODUCTION

Hair cells of the vestibular organs and cochlea transduce head movements, gravitational forces, and sound vibrations into electrical signals that are conveyed to the brain stem via the eighth cranial nerve. The physiology of hair cells has predominantly been studied in fish, amphibians, reptiles, birds, and rodents.[1-3] However, the upright posture and rich vocalizations of humans place demands upon our hair cells that differ from those of other animals. Understanding the human senses of balance and hearing requires understanding human hair cells.

Recently, we performed the first investigations into the physiology of human hair cells and found that on a single-cell level, they act quite similar to non-primate hair cells.[4] To do this study, we used the inner ear organs of patients undergoing lateral skull base tumor resection that required inner ear sacrifice. This is a select patient population in which hearing and vestibular sense cannot be preserved. In this report, we describe our patient population, our harvesting methodology, and the morphological characteristics of the harvested tissue. The ability to harvest living human hair cells is a powerful tool that will enhance the study of human inner ear diseases.

METHODS

Patient Population. Patients who required inner ear ablation at The Methodist Hospital Neurosensory Center were asked to participate in this study. The study protocol was approved by the Baylor College of Medicine Institutional Review Board, and informed consent from each patient was obtained after the nature and possible consequences of the surgery were discussed. Indications for lateral skull base surgery at our institution include the presence of an enhancing tumor as demonstrated by magnetic resonance imaging (MRI) with intravenous gadolinium contrast. Most patients who meet this requirement have unilateral vestibular schwannoma (incidence 1 in 100,000 per year).[5] We use the translabyrinthine approach in all cases of nonserviceable hearing or if the tumor extends into the cerebellopontine angle more than 1 cm (drilling out the semicircular canals and vestibule only). This may be extended to the transotic approach (drilling out the semicircular canals, vestibule, and cochlea) if the tumor diameter is greater than 3 cm.

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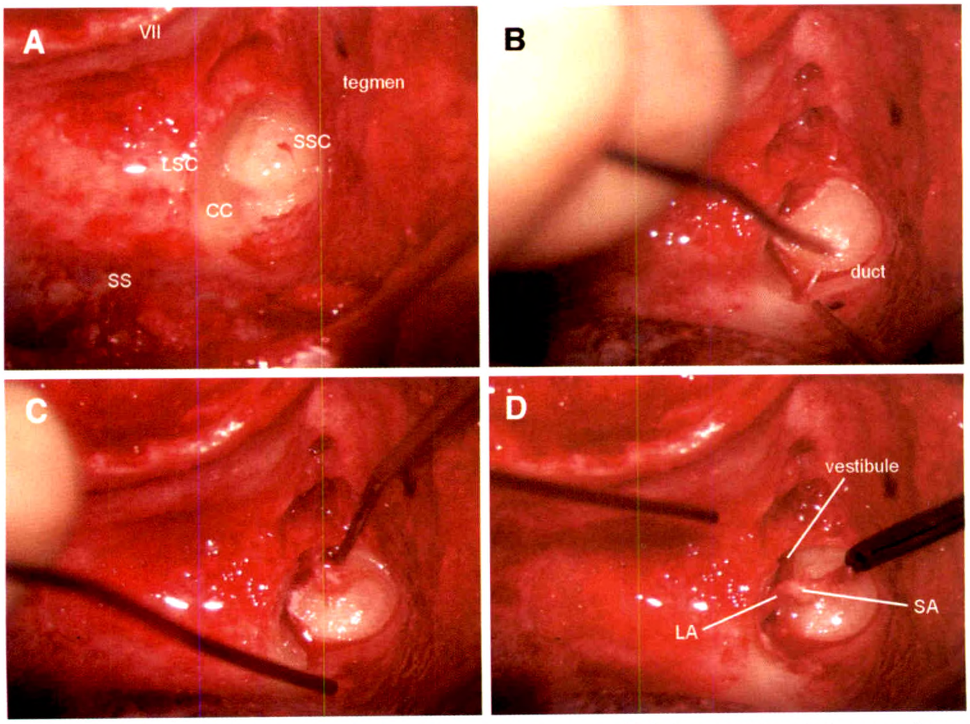

Fig 1. Technique of harvesting vestibular organs by translabyrinthine approach (left ear, with up being anterior and right being superior). A) Standard mastoidectomy with blue-lined superior semicircular canal (SSC), lateral semicircular canal (LSC), and common crus (CC). Also demonstrated are facial nerve (VII), sigmoid sinus (SS), and tegmen. B) Completely opened SSC, with membranous duct tented upward by suction tip. C) Membranous SSC duct and ampulla elevated out of bony labyrinth. D) Contents of vestibule pulled out along with SSC and LSC ampullae (SA and LA, respectively). This was done with cup forceps.

Surgical Technique. The translabyrinthine and transotic approaches for resection of a vestibular schwannoma have been described in detail.[6-10] We modified these techniques in an attempt to minimize trauma to the inner ear tissue. Since these specimens were quite small and delicate, careful exposure and dissection was critical. We used a Fisch otologic drill (Jedmed, St Louis, Mo) for the surgery. Sterile PhysioSol (Abbott, Chicago, Ill), a balanced salt solution with a pH of 7.4 and an osmolality of 294 mOsm/kg, was used as irrigation fluid during drilling. An operating microscope (M655, Leica, Allendale, NJ) with an attached 3-chip video camera (Storz, St Louis) was used for visualization and intraoperative photography. Facial nerve monitoring was performed throughout the harvesting procedure with 3-channel, free-run electromyography (Viking 4P, Nicolet Instruments, Madison, Wis). Inhalation agents were used for anesthesia.

To harvest the vestibular organs, we first performed a complete mastoidectomy with identification of the facial nerve. The superior and lateral semicircular canals were blue-lined to their ampullae (Fig 1A). Next, a diamond bur was used to carefully enter the labyrinth at the common crus. We unroofed the circumference of both bony canals, using care to preserve the membranous labyrinth (Fig 1B). The vestibule was then opened at the junction of the superior and lateral canal ampullae. We did not attempt to skeletonize and harvest the posterior semicircular canal ampulla, due to the overhang of the facial nerve. Next, we began harvesting the membranes of the inner ear. The membranous duct was our starting point, because it does not contain sensory epithelium. We used an excavator or a right-angled pick (not shown) to elevate the superior and lateral canal ducts out of the bony labyrinth (Fig 1C). This freed up tension on the ampullae and the membranes of the vestibule. Next, we elevated the superior and lateral canal ampullae out of the lumen of the bony canal, using blunt dissection to cut the nerve bundles. The contents of the vestibule were dissected out similarly (albeit blindly), by prying their membranes away from the bony wall. Finally, we grasped the semicircular canal duct with a cup forceps and attempted to pull the intact specimen from the wound (Fig 1D).

To harvest cochlear tissue via the transotic approach, we first performed a complete mastoidectomy with exposure of the middle ear, removal of the tympanic membrane, malleus, and incus, and preservation of the facial nerve (Fig 2A). Labyrinthectomy with harvest of the vestibular organs was then performed as described above. Next, we made 2 cochleostomies using a diamond bur at the promontory: one

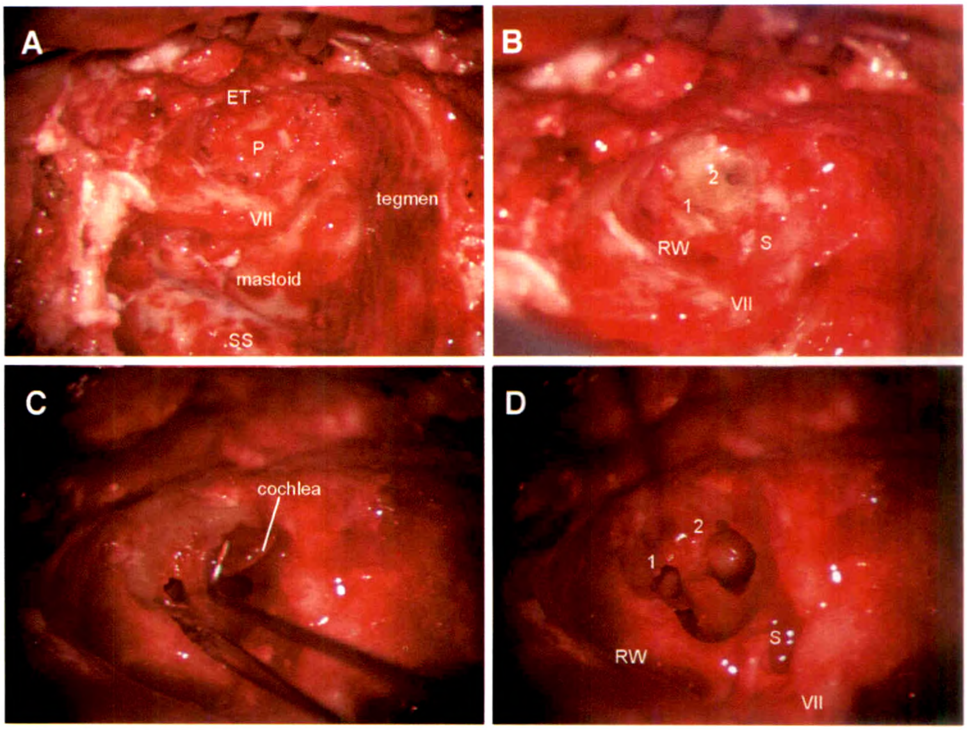

Fig 2. Technique of harvesting cochlea via transotic approach (left ear, with up being anterior and right being superior). A) Complete canal wall–down mastoidectomy with removal of incus and malleus. Landmarks include eustachian tube (ET), promontory (P), facial nerve (VII), sigmoid sinus (SS), and tegmen. B) Blue-lined first (1) and second (2) turns of cochlea. Round window niche (RW) and stapes (S) are visible. C) Dissection of cochlear second turn out of otic capsule with right-angled pick. Other instrument visible is suction tip. D) After harvesting was completed, empty cochlear cavity remained.

over the first turn and the other over the second turn (Fig 2B). Care was taken to preserve the spiral ligament. Wider exposure was performed by delicately curetting away as much surrounding bone as possible. We then used an excavator (not shown) or a right-angled pick to tease the spiral ligament away from the inner surface of the otic capsule. Blunt dissection was used to cut the modiolus at the base, and between the first and second cochlear turns (Fig 2C). The first and second turns of the cochlea were lifted out of the bony labyrinth individually, in an attempt to maintain the integrity of the modiolus, spiral ligament, and intervening organ of Corti. This left behind an empty cochlear cavity (Fig 2D).

The inner ear specimens were immediately placed into a sterile container containing PhysioSol. This was put on ice and taken to the laboratory, where the tissue was transferred into a nutrient-containing culture medium of L-15 (Gibco BRL, Gaithersburg, Md) with 5-mmol/L *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES, Sigma, St Louis), hereinafter referred to as our "standard" solution. The standard solution was at room temperature (22°C to 24°C), with a pH of 7.3 and an osmolarity of 320 mOsm/kg. Specimen transport and transfer into the standard solution was performed within 5 minutes of harvesting. A dissecting microscope (Wilde, Switzerland) was used in the laboratory to debride the vestibular organs. Photographs of the gross organs were taken with an upright microscope (Labophot, Nikon, Japan) with Plan 1.3 (Olympus, Japan) and Plan 4 (Nikon) objectives.

Vestibular Hair Cell Preparations. Vestibular hair cells were isolated from the semicircular canal organs, utriculus, and sacculus. To do this, we incubated the vestibular organs in standard solution containing papain (0.5 mg/mL, Sigma), L-cysteine (0.3 mg/mL, Sigma), and ethylene glycol-bis (*B*-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA; 1.2 mmol/L, Sigma) at 37°C for 35 minutes. The organs were then transferred back into normal standard solution, and the epithelia were mechanically dissociated with a human eyelash onto the glass bottom of a microwell Petri dish coated with poly-D-lysine (MatTek Corporation, Ashland, Mass). After allowing 10 minutes for the cells to settle, we began constant perfusion with fresh standard solution. Microscopic visualization was performed with a 100× oil immersion objective (Axiovert 35, Zeiss, Germany) and Nomarski optics.

In 4 specimens, vestibular hair cells were studied in situ in the utricular or saccular epithelium, with methodology first developed for mice.[11,12] Briefly, 2 glass fibers of roughly 50 μm in diameter were

CHARACTERISTICS OF PATIENTS UNDERGOING INNER EAR HARVESTING

Patient	*Age*	*Sex*	*Unilateral Weakness (%) Ipsi*	*Directional Preponderance (%) Contra*	*Other Vestibular Deficits*	*PTA Ipsi/ Contra (dB)*	*SD Ipsi/ Contra (PB_{max} % correct)*	*Tumor Diameter (cm)*	*Semicircular Canal Organs*	*Utriculi*	*Sacculi*	*Cochlea*
1	70	F	36	12	0	53/13	60/100	1.2	0	0	0	NP
2	45	M	72	22	0	41/10	64/96	2.7	2	0	0	NP
3	64	F	NP	NP	NP	105/33	0/52	2.0	0	0	0	NP
4	48	F	NP	NP	NP	68/3	0/92	3.5	1	0	0	Half turn 2
5	64	F	NP	NP	NP	53/20	0/100	2.0	2	1	0	NP
6	65	M	34	26	0	51/7	100/100	2.0	1	1	0	NP
7	45	F	100	36	0	23/10	NP	3.0	2	0	1	NP
8	41	F	67	43	1*	78/17	0/80	4.0	1	0	1	Complete turn 1; half turn 2
9	44	F	51	2	0	47/13	4/96	3.0	2	0	1	NP
10	54	M	NP	NP	NP	43/8	72/100	1.5	1	1	1	NP
11	61	M	5.6	5.6	0	16/13	92/100	1.6	1	1	0	NP
12	37	M	38	10	0	5/5	NP	2†	2	0	1	Half turn 1; complete turn 2
13	35	F	50	6	0	38/2	60/96	1.6	2	0	0	NP
14	58	F	NP	NP	NP	45/22	64/100	2.0	2	1	0	NP
15	50	F	NP	NP	NP	22/10	96/100	2.0	2	1	0	NP
16	63	M	NP	NP	NP	105/95	NP	0.8	2	0	1	NP
17	37	F	20	16	0	62/22	NP	3.1	NP	NP	NP	Complete turn 2
18	73	F	NP	NP	NP	60/27	68/100	1.8	0	1	0	NP
19	56	M	NP	NP	NP	13/13	NP	1.0	1	0	0	NP
20	66	F	25	14	0	43/43	72/92	1.3	1	1	0	NP
21	50	F	14	4	0	56/8	28/100	1.3	1	1	0	NP
22	68	F	NP	NP	NP	45/18	76/100	1.5	0	0	0	NP
23	70	M	3	5	0	80/37	52/76	1.5	2	1	1	NP
24	52	F	48	21	0	42/5	56/96	2.2	2	1	0	NP
25	38	M	NP	NP	NP	27/8	100/100	2.0	0	1	1	NP

PTA — pure tone average, SD — speech discrimination, NP — test not performed or harvesting not attempted.

*Left-beating nystagmus on oculomotor tests suggesting brain stem compression, which was verified by magnetic resonance imaging.

†Facial neuroma.

glued down flat at one end to a glass coverslip. The organs were laid flat on the coverslip, epithelial surface facing up, and held in place by the glass fibers. Constant perfusion of the tissue with fresh standard solution was performed. The in situ preparation was visualized under an upright microscope with a 40× water immersion objective (Axioskop FS, Zeiss) and Nomarski optics. Semicircular canal organs were not studied in situ, because their curved shape and greater thickness degraded visualization.

Cochlear Outer Hair Cell Preparation. Cochlear outer hair cells were studied with an isolated cell preparation developed for guinea pigs.[13,14] We cut the organ of Corti from the modiolus as one strip using a microscissors. This was placed in a microcentrifuge tube with standard solution containing trypsin (0.5 mg/mL, type III, Sigma) and incubated at 31°C for 5 minutes. Gentle trituration was performed to dissociate the cells. Isolated outer hair cells were plated and visualized as described for the isolated vestibular hair cells.

RESULTS

Patient Characteristics. Over the 10-month study period, every potential subject agreed to donate his or her inner ear organs. The study group (see Table) consisted of 25 patients (9 men and 16 women) who ranged from 35 to 73 years old (54 ± 12). (Note: all values in parentheses are mean ± SD.) All patients had a vestibular schwannoma located in the internal auditory canal or cerebellopontine angle, except for patient 12. This patient had a facial neuroma that ex-

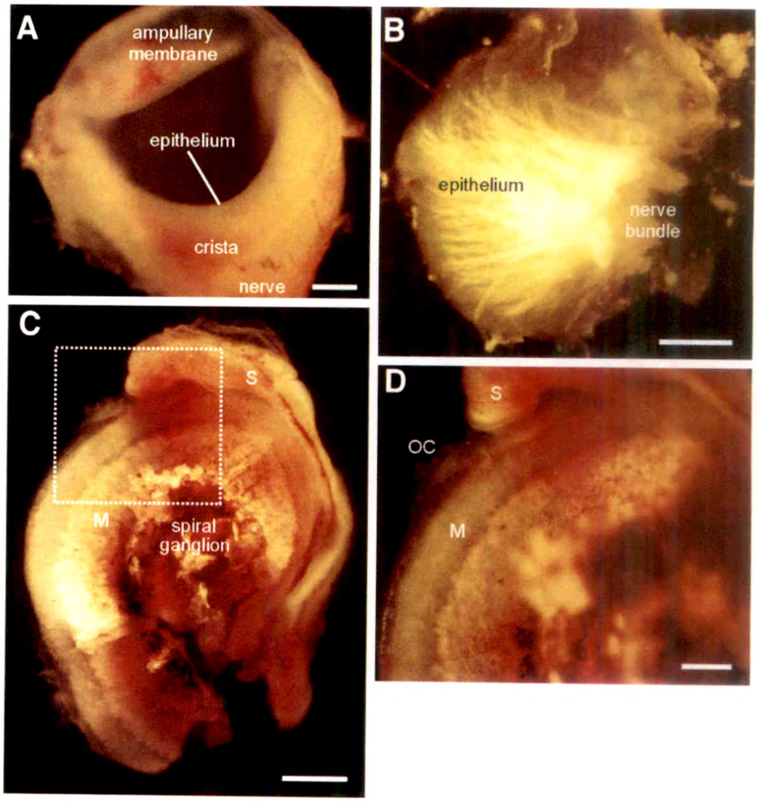

Fig 3. Human inner ear organs. A) Ampulla of semicircular canal organ after debridement of surrounding tissue. Opaque, saddle-shaped crista is inferior and appears undamaged. Vestibular hair cells lie in sensory epithelium on superior surface of crista. Afferent nerve fibers exit inferiorly from crista. Thin ampullary membrane is superior. No cupulae could be identified in any of our specimens, perhaps because they were not optically dense enough to be detected visually. Scale bar — 0.25 mm. B) Utriculus viewed from above, looking down onto its epithelial surface, after removal of otoconia. Myelinated afferent nerve fibers are visible as white streaks running below its epithelial surface, and parallel to it. Utricular nerve bundle is on right. Scale bar — 0.5 mm. C) Cochlear second turn. Half of spiral ligament (S) is missing. Spiral ganglion cells are in center of bony modiolus (M). Scale bar — 0.5 mm. D) Close-up of area of interest from C. OC — organ of Corti; scale bar — 0.25 mm.

tended from the horizontal segment of the facial nerve into the temporal lobe of the brain. Fourteen patients had preoperative vestibular testing, and they were found to have various degrees of peripheral vestibular dysfunction. Caloric testing of the vestibulo-ocular reflex demonstrated ipsilateral unilateral weaknesses ranging from 3% to 100% (40 ± 27) and contralateral directional preponderances ranging from 2% to 43% (16 ± 12). Values of greater than 20% are considered abnormal. Except for patient 8, there were no other significant findings noted with oculomotor testing, rotatory testing, positional testing, and posturography. Patient 8 had left-beating nystagmus superimposed on saccades, gaze nystagmus in the left gaze position, and nystagmus during smooth pursuit to the left. These findings suggested brain stem compression, which was verified by MRI.

Most patients demonstrated some degree of preoperative ipsilateral sensorineural hearing loss by comparison of their pure tone averages (PTAs; average of the pure tone thresholds at 500, 1,000, and 2,000 Hz). The ipsilateral PTAs ranged from 5 to 105 dB (49 ± 25), and the contralateral PTAs ranged from 2 to 95 dB (17 ± 18). There was usually also a loss of speech discrimination caused by the tumors. The ipsilateral maximum phonetically balanced (PB_{max}) word scores ranged from 0% to 100% (53 ± 35), while the contralateral scores ranged from 52% to 100% (94 ± 12). Tumor size was measured from MRI, and the largest diameters are presented in the Table. Sizes ranged from 0.8 to 4 cm (2.0 ± 0.8).

Harvesting Specimens. Twenty-one of the patients underwent a translabyrinthine approach with attempted harvest of the vestibular organs. The remaining 4 (patients 4, 8, 12, and 17) underwent a transotic approach with attempted harvest of cochlear tissue. We also attempted to harvest vestibular tissue from 3 of them (patients 4, 8, and 12). Altogether, tissue that contained hair cells was collected from 22 of the 25 patients (see Table).

An ampulla of a semicircular canal (Fig 3A) was harvested most frequently, and we preserved at least 1 canal organ in 19 patients. They were the easiest of the organs to harvest, because they could be directly visualized and completely exposed during the operation. This permitted extraction of the tissue from the surgical wound with minimal trauma to the sensory epithelium. A cupula was never appreciated in any of the specimens; it is a nearly translucent structure and would be difficult to distinguish during the dissection process. The utriculus (Fig 3B) was harvested from 12 patients, and the sacculus was harvested from 8 patients. They are thin, flat structures that require more delicate handling than do the semicircular canal organs. Their corresponding otoconial layers were most often found loose within the tissue specimen, rather than appropriately attached to the surface of the epithelium. Presumably, the otoconial layer detaches easily.

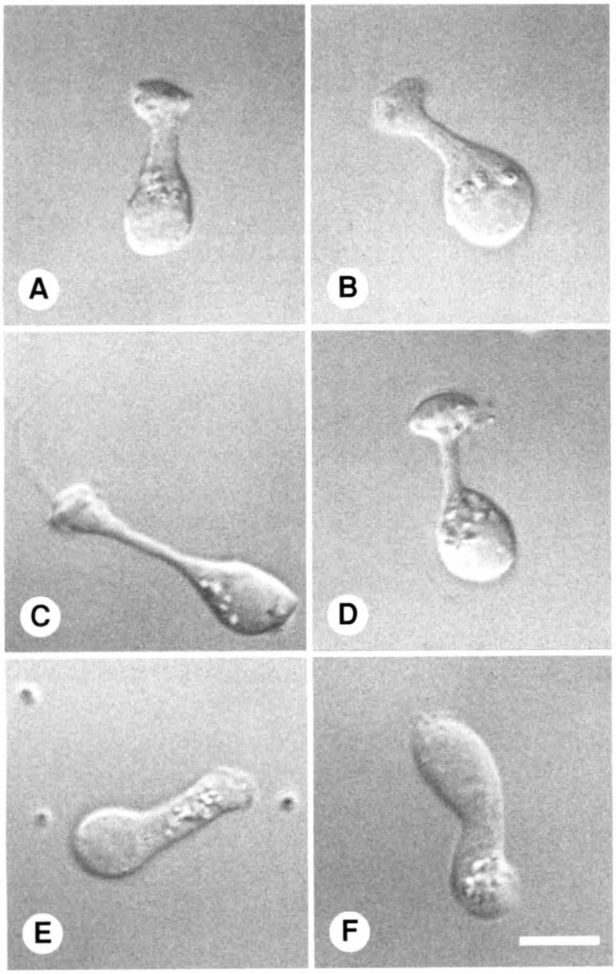

Fig 4. Vestibular hair cells from human semicircular canal organs. A-D) Type I cells (found only in amniotes) have constricted neck just below cuticular plate. Note presence of long, bent stereocilia of cell in C. None of other cells had stereocilia. E,F) Type II cells (found in all vertebrates) with cylindrical shape. Neither of these cells had stereocilia. Scale bar — 10 μm.

Cochlear harvesting was attempted from 4 patients (Fig 3C,D). The cochlea proved quite difficult to harvest intact, so we instead focused on trying to preserve 1 turn at a time. At least a portion of the second turn of the cochlea was harvested from each patient. We could also preserve a segment of the first turn in 2 patients; we could not harvest any tissue at all from the apex of the cochlea. Under the dissecting microscope in the laboratory, we noted that in sites in which the spiral ligament was not attached to the modiolus, the organ of Corti was usually missing. Hence, careful surgical technique to preserve these microscopic structures is of paramount importance. We did not attempt to visualize the intact organ of Corti in situ, so its architecture (including the basilar membrane and the tectorial membrane) was not evaluated.

Morphological Features of Hair Cells. Under the light microscope, vestibular hair cells displayed a standard morphology based on cell type.[15] Both type I and type II cells were about 20 to 25 μm in length, with stereocilia at the apex and a nucleus at the base. Type I cells (Fig 4A-D) had a constricted neck just below the apex, while type II cells (Fig 4E,F) had a more cylindrical shape. We typically could isolate 10 to 15 healthy-appearing cells per organ using the isolated cell preparation. Most cells had stereocilia that were swollen, splayed, or missing. The in situ preparation of the utriculi and sacculi demonstrated damage to all stereocilia from all specimens uniformly (n = 4). This occurred even in the few specimens in which the otoconial layer was still attached to the epithelium and was removed by appropriate enzymatic techniques in an attempt to minimize damage to the stereocilia.[11,12]

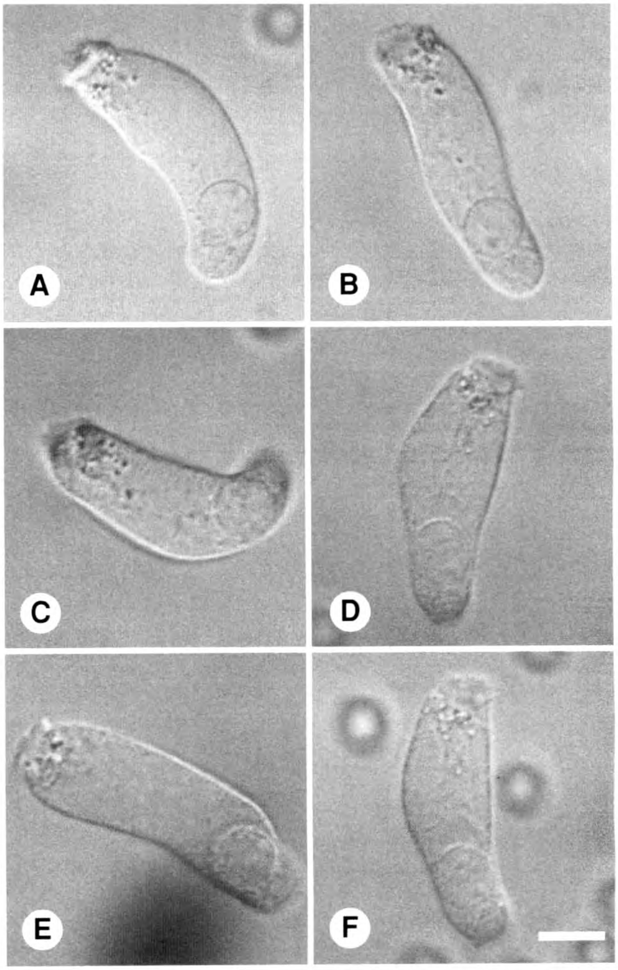

Fig 5. Various (A-F) cochlear outer hair cells from second turn. They are cylindrical and have basally located nucleus. None of these cells had stereocilia present. Scale bar — 10 μm.

Outer hair cells from the cochlea ranged from 30 to 70 μm in length (Fig 5). The condition of their stereocilia varied from normal to missing. They had a prominent nucleus at the base. Their cytoplasm was clear, with no brownian motion of cytoplasmic particles. We could usually isolate 5 to 10 healthy-appearing cells per patient, depending on the amount of intact organ of Corti that was harvested. Cochlear inner hair cells were not observed in the dissociated material, as is typical of rodent preparations with the same dissociation technique.[13,14] In summary, the human hair cells we harvested using these methodologies appeared healthy under microscopic visualization and had the standard morphologies of type I and type II vestibular hair cells and cochlear outer hair cells, respectively. These cells could be studied in vitro for approximately 4 hours after they were removed from the patient before cell death, similar to hair cells of other mammals.

Surgical Complications. There were no complications from the harvesting procedure. Blood loss during the harvest was insignificant. The bone surrounding the facial nerve within the mastoid was never encroached on during any harvest, and there was no stimulation noted on facial nerve monitoring. The harvesting procedure did not affect the subsequent surgical approach to the tumor or enlarge the post-resection temporal bone defect.

The time required to harvest the tissue was minimal. Since resections of lateral skull base tumors tend to be lengthy procedures in their own right, we were particularly sensitive to elongating the anesthetic time of our patients. Retrieving the semicircular canal organs added only 5 to 10 minutes' time to the operation. Since the vestibule was open during this process, the utriculus and sacculus could be harvested within the same time period. Cochlear harvesting took longer, adding approximately 20 minutes to the length of the procedure.

DISCUSSION

Hair Cell Viability. We used no patient selection criteria for the study. Consent was requested from all patients, and all agreed to the harvesting procedure. The most frequent tumor in our patient population was a vestibular schwannoma. These tumors can encroach upon the labyrinthine vessels that supply the inner ear organs, and we were concerned about whether the hair cells we harvested would represent "normal" hair cells. In fact, many of our patients had substantial balance and hearing deficits preoperatively. Clearly, patients with normal or near-normal vestibular tests or audiograms *must* have functioning vestibular or cochlear hair cells, respectively. However, because the presence of a vestibular schwanomma can selectively block eighth nerve conduction, patients with abnormal test results may still have normal hair cells.

Even though sensory deficits may exist, this harvesting technique tends to select for and preserve the remaining viable hair cells. All human hair cells that we performed electrophysiological studies on had membrane ion channel physiology (in vestibular hair cells) and electromotile responses (in cochlear outer hair cells) similar to those of hair cells of other animals, particularly other mammals.[4] While our sample size of 25 patients is too small to make meaningful correlations between preoperative test results and hair cell physiology, we can have confidence that this harvesting technique is a relatively safe and effective means of collecting viable vestibular and cochlear hair cells from humans.

Trauma of Harvesting. In our experience, the high incidence of stereociliary damage in isolated human vestibular and cochlear hair cells is typical of that seen with hair cells isolated from other animals. In contrast, the consistent injury to all stereocilia in the utriculi and sacculi in situ preparations is not typical of similar preparations in other animals. Since, in vivo, the otoconial layer is tethered to the tips of the stereocilia, detachment of the otoconial layer probably is responsible for the damage to the stereocilia. This may have been due to mechanical trauma during the actual harvesting procedure or due to vibration injury during the drill-out of the temporal bone. We feel this latter possibility is unlikely, however, since vestibular deficits and sensorineural hearing loss following mastoidectomy and stapedectomy do not appear to be linked to drilling.[16,17]

Potential Applications. There are several potential applications of human vestibular and cochlear tissue that could prove to be quite beneficial. For example, further physiological data could be collected from single hair cells (such as voltage-current relationships, mechano-electrical transduction currents, neurotransmitter responses, etc). These data could then be correlated with data from noninvasive tests of inner ear function such as oculomotor testing, vestibulo-ocular reflex testing, positional testing, posturography, galvanic stimulation, pure tone thresholds, and otoacoustic emissions.[18-25] By also involving data from human psychophysical studies, a new approach for the study of human inner ear function is possible — a way to relate cellular function to human perception. Additionally, hair cell regeneration studies could be performed directly with human tissue. This has been done once with adult human vestibular organs[26]; however, improvements in harvesting technique could make this commonplace. Harvested human inner ear tissue will be an important testing ground during the development of therapeutic agents designed to stimulate hair cell regeneration or repair. Also, our understanding of the genetic basis of balance and hearing disorders might be advanced by the use of human tissue. Currently, most screening strategies use inner ear complementary DNA (cDNA) libraries created from rodent animal models to probe for possible sites of mutagenesis.[27,28] A more representative cDNA library could be formulated from human inner ear tissue. This has been attempted on postmortem human fetal tissue[29]; however, there may be circumstances in which a cDNA library created from adult, fully developed hair cells would be preferable. In conclusion, the overall goal of most biomedical basic science research is to further understanding of health and disease in human beings; for otologic research efforts, this can be furthered by directly using human inner ear tissue.

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RESULTS OF TYMPANOPLASTY IN CHILDREN AFTER 15 TO 27 YEARS

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Results of tympanoplasty in children with noncholesteatomatous chronic otitis media are presented in a 16- to 27-year follow-up. During the 13-year period from 1968 to 1980, 116 children (124 ears) were operated on, and they were submitted to several follow-ups with audiometry and otomicroscopy. The attendance at the last follow-up was 70%, and the minimum time since surgery for these patients was 15 years. All dry ears were operated on transcanally with a fixed ear speculum, without any lateral incision of the ear canal skin. Cumulatively, in total, 14 ears (11%) had reperforation: 7 ears early and 7 ears late. At 6 months, there were 6% early reperforations; some were surgically closed and some reappeared later, even after several years, as late perforations. At 2 to 15 years of follow-up, there were 4% reperforations, and at 16 to 27 years, 6%. Hearing was good and stable. The results were the same in ears operated on at the ages of 2.5 to 7 years and 8 to 14 years, as well as with preoperatively positive and negative Valsalva maneuvers. In total, 14% of ears were revised during the entire observation period. It is concluded that transcanal tympanoplasty, even in young children, has good long-term stability and can definitively and permanently solve the problem of noncholesteatomatous chronic otitis in children.

KEY WORDS — chronic otitis media, long-term results, myringoplasty in children, tympanoplasty in children.

INTRODUCTION

The goal of this study is to present and analyze the results of surgery for noncholesteatomatous chronic otitis media in children in a 16- to 27-year follow-up. All children that were operated on during the period of 1968 to 1980 are now adults, and it is important to investigate how stable the surgical results have become, and how much additional surgery was required to achieve an optimal and permanent cure or a stable suboptimal outcome. Such systematic long-term studies have not been performed previously, maybe because of the large dropout rates of teenaged and young adult patients.

There has been an ongoing debate on the indications for tympanoplasty in children and on the question of when to close the eardrum perforation.[1-10] Some surgeons have been restricted to waiting until the child is more than 10 years old,[1] some prefer to operate on children at school age, and some recommend operation unrelated to age.[2,5-10]

TABLE 1. BASIC DISEASES, METHODS, AND APPROACHES APPLIED IN VARIOUS GROUPS

		Methods and Approaches		
			Retroauricular Mastoidectomy and Tympanoplasty	
Disease Groups	*Total Ears*	*Transcanal Tympanoplasty Only*	*Canal Wall Up*	*Canal Wall Down*
Sequelae to chronic otitis	94	94		
Active chronic otitis	22	3	7	12
Adhesive otitis	8	8		
Total (ears)	124	105	7	12

MATERIAL AND METHODS

The basic material was exactly the same as presented in 1986.[5] It comprised 124 ears from 116 children, ages 2.5 to 14 years, operated on during the

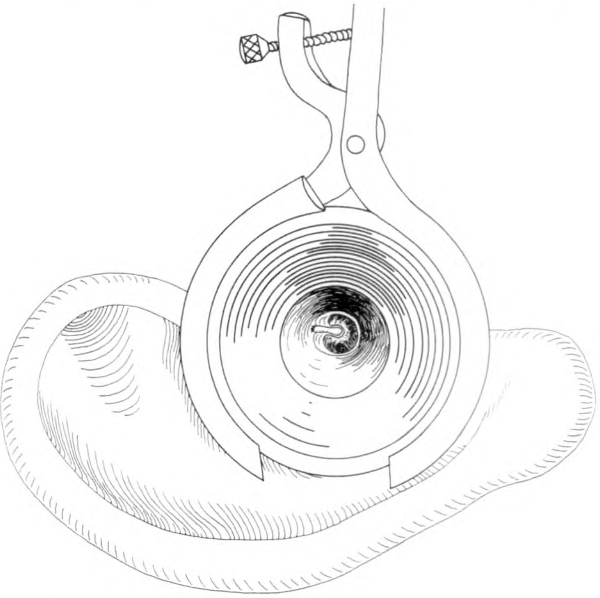

Fig 1. Transcanal approach with fixed ear speculum without any lateral incision of canal skin. Speculum is fixed with Richards speculum holder.

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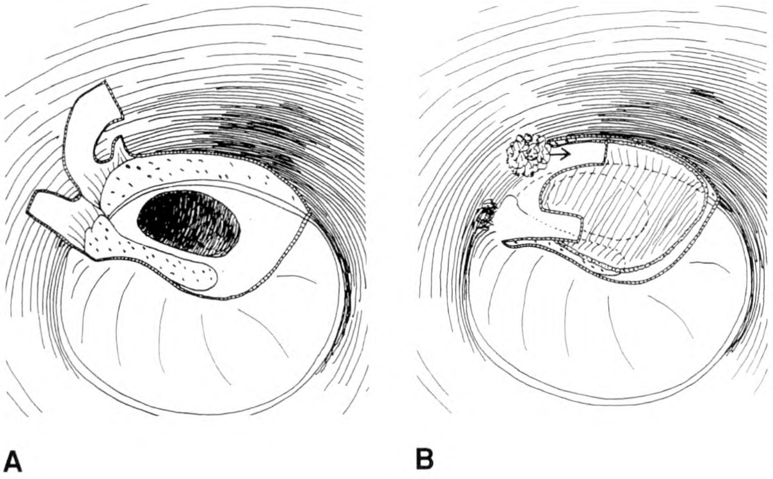


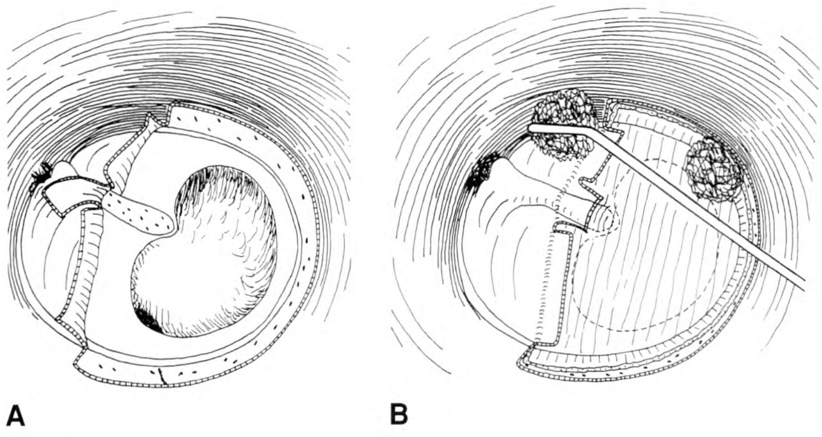
Fig 2. Onlay fascia grafting in anterior perforation. A) Malleus epithelial flap and anterosuperior skin flap are elevated, and keratinized epithelium around perforation is removed. B) After placement of fascia onto denuded part of eardrum and ear canal, flaps are replaced.

13-year period from 1968 to 1980. At the time of surgery, 26 ears were from children 2.5 to 7 years old, and 98 ears from children 8 to 14 years old. The ossicular chain was intact in 86 ears; in these ears a myringoplasty or tympanoplasty type I was performed. In 32 ears, the long process of the incus was resorbed, and a tympanoplasty type II with interposition of an autogenous incus was made. In 6 ears, only the stapedial arch was missing, and a tympanoplasty type III was performed with an autogenous incus used as a columella. The demographic data and the surgical methods used were described previously[5] and will only be summarized briefly.

The diagnosis in most children (94 ears) was of sequelae of chronic otitis media with eardrum perforation, and most of the ears were dry (Table 1). The indications for transcanal myringoplasty (Fig 1) were closure of the perforation, hearing improvement, and/or to stop recurrent discharge or ingrowth of keratinized epithelium from the posterior perforation. In 22 ears the preoperative diagnosis was active chronic (granulating) otitis media with chronic ear discharge, and the main indication for surgery was to make the ear dry. Canal wall up mastoidectomy with tympanoplasty at the same stage was performed in 7 ears, canal wall down mastoidectomy with cavity obliteration in 12 ears, and tympanoplasty without mastoidectomy in 3 ears. In the 1960s and early 1970s, our method of choice in active chronic (granulating) otitis media resistant to conservative treatment was canal wall down mastoidectomy with obliteration and tympanoplasty. In the following years, the method of choice for such conditions was canal wall up mastoidectomy with tympanoplasty (Table 1). In 8 ears the posterosuperior part of the eardrum was heavily retracted and adherent to the medial wall of the tympanic cavity. The diagnosis was adhesive otitis media, treated by transcanal tympanotomy, removal of the retracted membranes, and underlay fascia grafting.

The perforations were total in 22%, anterior in 20%, inferior in 21%, and posterior in 38% of cases. All procedures were performed in 1 stage — the 105 myringoplasties or tympanoplasties through the transcanal approach with a fixed ear speculum, and the 19 mastoidectomies with a retroauricular approach (Table 1). Onlay fascia grafting was applied for anterior (Fig 2), inferior (Fig 3), and total perforations (Fig 4).[11] Posterior perforations were closed with transcanal myringoplasty or tympanoplasty and un-

Fig 3. Onlay fascia grafting in inferior perforation. A) Incision is made 1 mm lateral to annulus, and epithelium from inferior part of eardrum is removed. Superiorly, small epithelial flaps are created. B) After placement of fascia onto denuded annulus and drum remnant, epithelial flaps are placed. Fascia is fixed with Gelfoam balls.



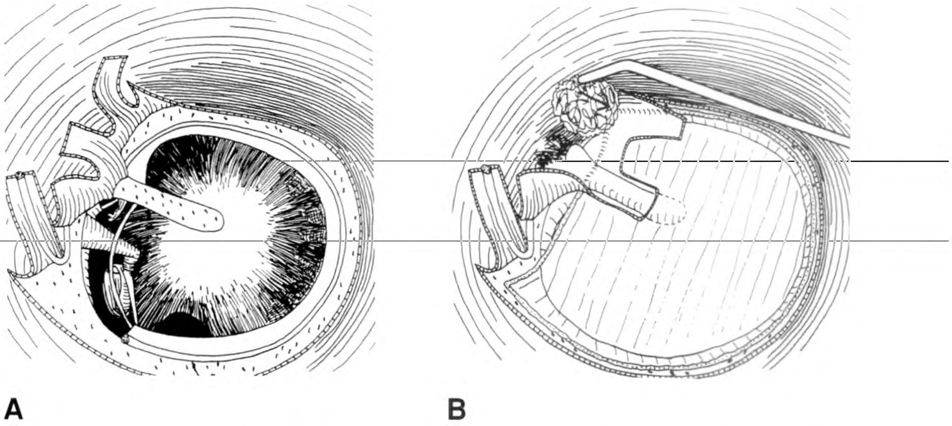


Fig 4. Onlay fascia grafting in total perforation. A) Ear canal skin is incised 1 mm lateral to annulus all around. Posterosuperior epithelial flap, malleus flap, and anterosuperior flap are elevated. B) Perforation is covered with fascia, and elevated skin flaps are replaced and fixed by Gelfoam balls.

derlay fascia grafting (Fig 5).[11]

The fascia was held in place by Gelfoam balls placed in the ear canal, and the ear canal was filled with 1-cm gauze, moistened with hydrocortisone-Terramycin ointment, for 3 weeks.

Follow-up. After the gauze was removed, the children were regularly followed up with audiological and otomicroscopic checkups during the first year, making a 100% 1-year follow-up. During the long observation period, we have performed several systematic follow-up investigations, asking the patients to come to the hospital to be evaluated with audiological tests, tympanometry, and otomicroscopy by otosurgeons. At 2 years postoperatively, the follow-up was 98% (Table 2). At the last evaluation, the follow-up was 70%, with a minimum observation time of 15 years and a maximum of 27 years. Eighty-eight percent of ears had a follow-up of 5 years or more, and 70% of ears, 10 years or more (Fig 6).

The reasons for nonattendance at the last follow-up in 1995-96 were as follows: died of other causes (2), immigrated (9), inability to locate (14), living far away (5), and failure to appear (15). At the last follow-up of the 2 children who died, performed at 6 and 13 years, respectively, after surgery, the conditions of the ears were normal.

Demographically, as well as in relation to the 1-year and 2-year results, the 45 ears from the nonattenders did not differ from the ears of the attenders.

RESULTS

Reperforations. Among 124 ears with perforations, 7 (6%) had an early first postoperative perforation (Table 2). The early perforations were presumably due to failure at surgery; they appeared early in the postoperative period. During the period of up to 2 years postoperatively, 6 perforations were surgically closed, but in 1 of these ears the perforation reappeared a second time, resulting at the end of the second postoperative year in 2% perforations. During the 2- to 15-year period, first reperforations occurred in 4 new ears, resulting in a total of 4% reperforations. During the period of 16 to 27 years after the operation, 3 new ears became reperforated for the first time, and in 3 ears, reperforation occurred a second time after a previously surgically closed reperforation, making a total of 6% reperforations at the latest follow-up.

Cumulatively, in total, 14 ears (11%) had a reper-

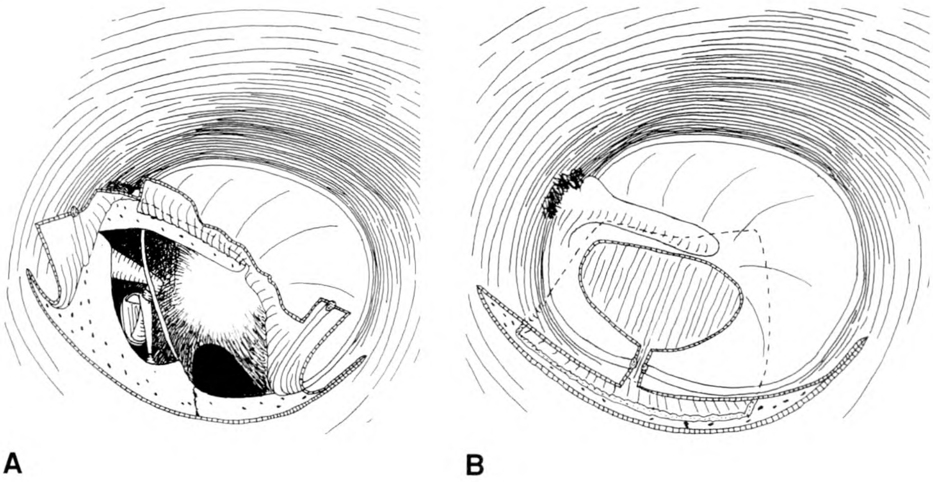


Fig 5. Underlay fascia grafting in posterior perforation. A) Skin is incised 3 mm lateral for annulus, and annulus and drum remnant are elevated. B) Fascia is placed under edges of drum, and skin flaps are replaced.

TABLE 2. NUMBER OF EARS WITH REOPERATIONS DURING VARIOUS FOLLOW-UP PERIODS

		Reperforations			*Perforations at End of Period*	
Follow-up Period	*No. of Ears*	*First*	*Second*	*Closed Surgically*	*No.*	*%*
0 to 6 mo	124	7		0	7	6
6 mo to 2 y	124		1	6	2*	2
2 to 15 y	110	4		1	4†	4
16 to 27 y	79	3	3	3	5	6
Total		14	4	10		

*One lost to follow-up after 2 years.

†Two lost to follow-up after more than 8 years.

foration (Table 2). In 7 ears the perforation appeared early, and in the remaining 7 ears, late.

Ears operated on in children younger than 8 years had 8% reperforations, in contrast to 12% in ears operated on in children 8 to 14 years old. In 62 ears in which the Valsalva maneuver was positive, the reoperation rate was 16%, and in 57 ears in which it was negative, the reoperation rate was 7%. At 2 to 15 years, evaluation showed a type A tympanogram in 70% of the ears.[5] At 16 to 27 years, 33% were of type A, 47% of type C1, 7% of type C2, and 13% of type B, but in this the 6% of reperforations were included.

Revisions. In total, 17 ears (14%) were revised during the entire postoperative period, 10 (8%) because of reperforation and 7 (6%) for other reasons (Fig 7). Among the 10 ears with revision because of reperforation (Fig 7), re-reperforation occurred in 4 ears. One was revised twice and another 3 times; in both, the perforations were closed at the last evaluation.

The indications for revisions that were done for reasons other than perforations were poor hearing, ear discharge, and attic cholesteatoma appearing some years after myringoplasty (Fig 7). One ear with hearing loss and 1 ear with attic cholesteatoma were again reoperated, resulting in a total of 22 (18%) revisions during the entire postoperative period.

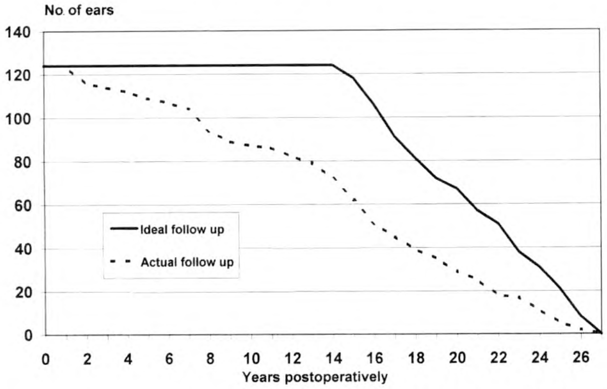


Fig 6. Ideal and realistic postoperative follow-up of 116 children during postoperative period of maximum 27 years.

Hearing. The hearing of the entire series, evaluated with various methods in the frequency range of 500 to 2,000 Hz, was evaluated preoperatively and at various postoperative periods (Table 3). The hearing testing was carried out in sound-attenuated audiometric test rooms according to International Standards Organization standards.

Absolute hearing, expressed as pure tone average for frequencies 500, 1,000, and 2,000 Hz (PTA), was good 3 to 15 years postoperatively, with 74% of ears within 20 dB; it dropped to 69% in the 16- to 27-year postoperative period. The speech reception threshold was within 20 dB in 74% of the ears at the last evaluation. The air-bone gap was within 10 dB in 45% of the ears, and within 20 dB in 81%. The reason for the relatively low percentage of complete air-bone gap closure is the entirely normal bone conduction. The hearing gain method expressed relatively small gains, mainly because the preoperative hearing was relatively good in some types of perforation, particularly in anterior and inferior perforations. Long-term stability, expressed as the mean of the individual absolute hearing (PTA; Fig 8) and as the mean air-bone gap (Fig 9) at various postoperative periods, was good, with practically the same hearing 20 years after the tympanoplasty. Tympanoplasty type I with an intact ossicular chain achieved the best hearing, but tympanoplasty type II with incus interposition also achieved good hearing up to 10 years after tympanoplasty. However, the latter had deteriorated slightly at the last reevaluation (Figs 8 and 9).

The long-term stability of hearing in ears with an intact ossicular chain and a preoperatively negative Valsalva maneuver was as good as that in ears with a positive Valsalva maneuver. The preoperative hearing was poorer in ears with a negative Valsalva maneuver (Fig 10). In ears with type II tympanoplasty,

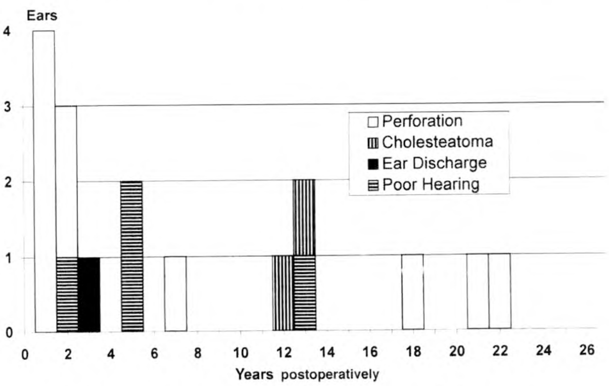


Fig 7. Number of ears with revisions, and time and reasons for first revisions.

TABLE 3. HEARING PREOPERATIVELY AND AT VARIOUS POSTOPERATIVE INTERVALS EXPRESSED WITH SEVERAL EVALUATION METHODS AS PERCENTAGE OF EARS AT CERTAIN LEVELS

			Absolute Hearing (%)		*SRT (%)*		*Air-Bone Gap (%)*			*Hearing Gain (%)*				
	Years Postop	*No. of Ears*	*0-20 dB*	*0-30 dB*	*0-20 dB*	*0-30 dB*	*0-10 dB*	*0-20 dB*	*0-30 dB*	*>20 dB*	*>10 dB*	*1-10 dB*	*No Gain*	*Deteriorated*
Preop		124	19	43	27	56	5	27	60					
Primarily	1	124	64	87	81	90	48	80	93	27	71	20	4	5
Late	3-15	110	74	88	76	88	56	88	97	41	71	18	6	5
Very late	16-27	79	69	78	74	86	45	81	90	23	49	29	12	10

SRT — speech reception threshold.

the hearing was poorer in ears with a preoperatively negative Valsalva maneuver, mainly because most of the ears with adhesive otitis belonged in this group (Fig 11).

Hearing in ears operated on at ages 2.5 to 7 years was not significantly poorer than in ears operated on at ages 8 to 14 years (Table 4; $p > .05$, χ^2 test). The recurrent perforations were equally distributed among all ages (Fig 12).

DISCUSSION

Even though there is a gap between the ideal and actual follow-up curves (Fig 6), the dropouts presumably have not influenced the general conclusions. The primary results and the results at the previous follow-ups of the 45 nonattenders did not differ from the results of the attenders. Analyses of the primary results of the 14 nonattenders at the evaluation in 1983-84 showed no difference between the attenders and the nonattenders.[5]

The long-term stability of tympanoplasty in children is good in adulthood and will presumably remain good with increasing age. However, there are no other publications with as long a postoperative observation time as this series, and most published series have a mean observation time of less than 5 years. The long observation time illustrates that some few ears will develop late reperforations (Table 2) and may develop attic cholesteatoma after closure of the perforation (Fig 7).

Results were as good in children operated on at the younger ages (2.5 to 7 years) as in those operated on at older ages (8 to 14 years) and support our previous conclusion,[5] based on the same material, that young children can be operated on with success if the surgery is indicated and desired. As Fig 12 illustrates, the reperforations were equally distributed among all ages, and another cutoff, eg, between 8 and 9 years, or calculation of a correlation coefficient gives no significant differences, partly because of the small size of the series. Several other reports support this view.[2,8-10] Some authors achieved poorer results in younger children[12-14] and recommend postponement of the surgery until 8 years of age.

Several factors support the assumption that a successful tympanoplasty in children presumably prevents deterioration to a more severe stage of noncholesteatomatous chronic otitis media.

First, the ossicular chain was, in this series, intact in a high percentage of ears (69%). The long process

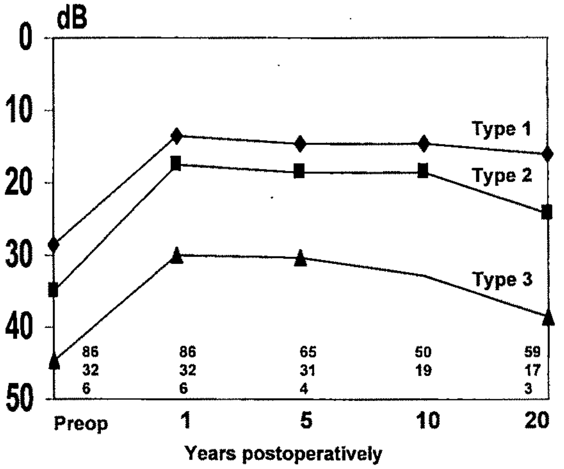


Fig 8. Long-term stability of mean absolute hearing (pure tone average) in various tympanoplasty types. Number of ears with types 1, 2, and 3 at various postoperative periods.

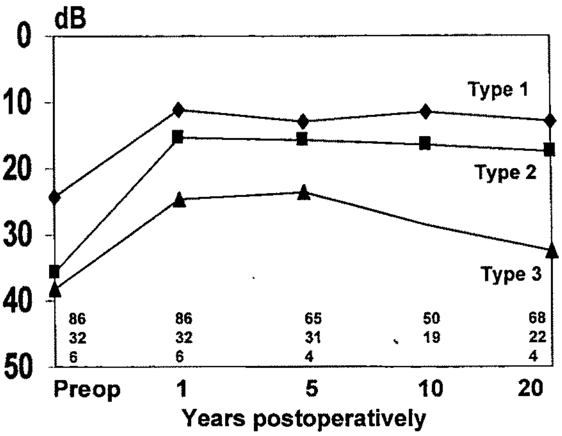


Fig 9. Long-term stability of mean air-bone gap in various tympanoplasty types. Number of ears with types 1, 2, and 3 at various postoperative periods.

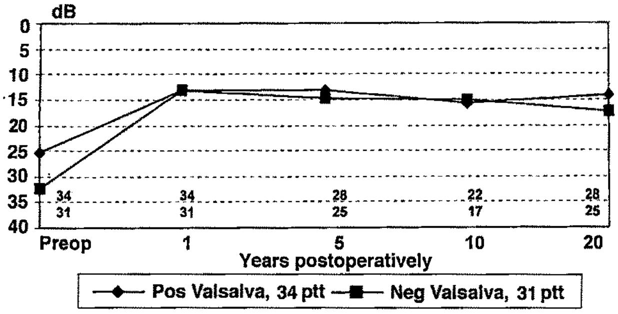


Fig 10. Long-term stability of mean absolute hearing (pure tone average) in ears with intact ossicular chain in relation to preoperative Valsalva maneuver.

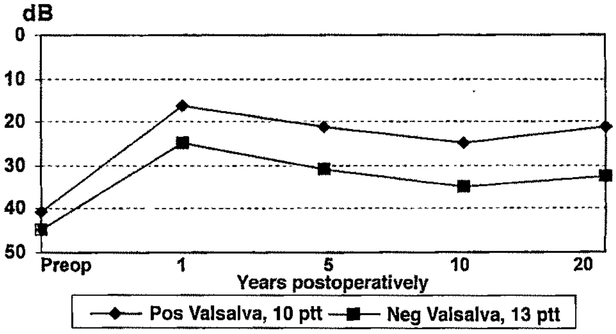


Fig 11. Long-term stability of mean absolute hearing (pure tone average) in ears with defective long process of incus and type 2 tympanoplasty in relation to preoperative Valsalva maneuver.

of the incus was defective in 26%, and the stapedial arch in 5% only. In a corresponding material of 774 adults with noncholesteatomatous chronic otitis, living in the same region and operated on during the same period, the condition of the ossicular chain was significantly poorer (χ^2 test, $p < .001$), with 45% with an intact chain, 46% with a stapes present with a long process of the incus defect, and 9% with the stapes absent. One cannot exclude the possibility that the presented pediatric series, if not operated on, would have deterioration in later childhood or adulthood and present worse ossicular damage. Practically all published surgical series of children with noncholesteatomatous otitis are dominated by ears with an intact ossicular chain.[1-10,12-20] After closure of the perforation, there has been no deterioration of the ossicular chain in this series during the long observation period, and the hearing has been surprisingly stable.

Second, myringosclerosis was a very common finding in the remaining part of the eardrum, but it was seldom as thick and severe as seen in the adult series of noncholesteatomatous chronic otitis, where 0.5- to 1-mm-thick plaques with ossifications were not uncommon. Again, a progression of myringosclerosis is possible if the perforation is not closed in childhood. After the closure of perforations in this series, we did not observe any progression of myringosclerosis. Before and during the surgery in several ears, infection and granulation formation on the lateral surface of the eardrum remnant were observed. These granulations can be the direct cause of myringosclerosis formation, leading to increased thickness of myringosclerosis over the years.

Third, recurrent infection in an ear with perforation in childhood may cause the formation of granulations in the tympanic cavity, which may be epithelialized by ingrowth of the keratinized squamous epithelium, representing another possibility for deterioration. Such ingrowth is in accordance with the immigration theory of cholesteatoma formation.

Tubal patency improved after myringoplasty, allowing normalization of the middle ear mucosa. This is indicated by the high take rate of ears with a negative preoperative Valsalva maneuver, and by the equally good postoperative hearing in patients with a preoperatively negative or positive Valsalva maneuver (Fig 10). In fact, we expected that a negative preoperative Valsalva maneuver would have some influence on the postoperative results, as was the case in our series of adults with noncholesteatomatous chronic otitis media.[21] The preoperative negative Valsalva maneuver improved in 82% of children.

Hearing and middle ear ventilation were surprisingly good at the last reevaluation, definitively indi-

TABLE 4. PREOPERATIVE AND POSTOPERATIVE ABSOLUTE HEARING AND AIR-BONE GAP IN 26 EARS OPERATED ON AT AGES 2.5 TO 7 YEARS COMPARED TO 98 EARS OPERATED ON AT AGES 8 TO 14 YEARS

		Years Postop	*No. of Ears*	*Absolute Hearing (%)*		*Air-Bone Gap (%)*	
				0-20 dB	*0-30 dB*	*0-10 dB*	*0-20 dB*
2.5-7 years (N = 26)	Preop		26	19	50	0	27
	Primarily	1	26	65	88	58	81
	Late	3-15	25	80	88	64	88
	Very late	16-27	23	65	74	39	74
8-14 years (N = 98)	Preop		98	19	41	6	27
	Primarily	1	98	65	87	46	81
	Late	3-15	85	72	88	54	89
	Very late	16-27	56	70	79	46	81

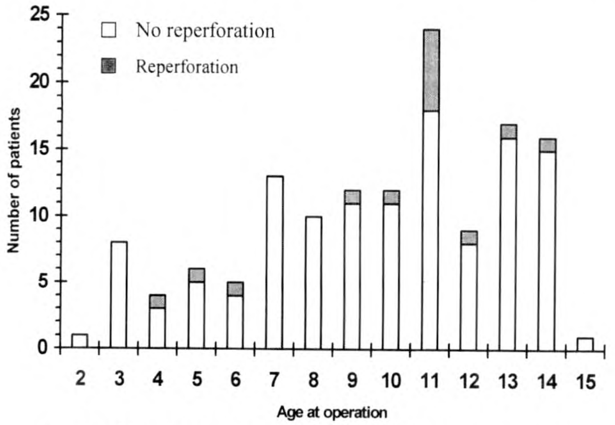


Fig 12. Number of children operated on at various ages and number of corresponding reperforations. Highest number of reperforations appeared after operation at age 11 years.

cating the permanent cure of the middle ear cleft in the vast majority of cases.

As shown in Table 2, there were a few ears with late perforations in connection with acute otitis media, which did not heal spontaneously. Appearance of the late perforations is understandable because of the preoperative atrophy of the drum remnant and/or atrophy of the fascia graft. We were able to close all 6 late perforations by revision surgery, but 4 ears were reperforated 1 to 10 years after the revision, in the form of new small late perforations. It is a matter of individual judgment, in accordance with patient's desire, whether such reperforations should be repeatedly closed.

We have performed nearly all operations via a transcanal approach with a fixed ear speculum, without any lateral incision of the ear canal. The high take rate and few revisions indicate that this is nearly always possible. The important factor of transcanal surgery in children is that there is no need for aftercare at all, and no fear of any granulation formation in the ear canal. We can strongly advocate this approach as well as onlay fascia grafting in anterior, inferior, and total perforations. In most operations, the mucosa of the tympanic cavity is not touched at all, and very little Gelfoam is placed into the tympanic cavity.[1]

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RESISTANT BACTERIA IN MIDDLE EAR FLUID AT THE TIME OF TYMPANOTOMY TUBE SURGERY

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This study was performed to determine the prevalence of resistant *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* isolated from middle ear fluid of children undergoing placement of ventilation tubes. The extent of resistance to commonly prescribed antibiotics and the risk factors associated with this resistance were also examined. Children who had fluid present in their middle ears at the time of ventilation tube placement from May 1996 to May 1997 were included in the study. Middle ear fluid was plated onto culture media in the operating room, and antimicrobial resistance of cultured organisms was ascertained. Risk factors for this resistance were determined from the medical history and analyzed. Cultures of 244 patients (355 ears) were positive for organisms in 29.6%. Penicillin resistance was found in 38.2% of *S pneumoniae* cultures. β-Lactamase production was found in 65.1% and 100% of *H influenzae* and *M catarrhalis* specimens, respectively. Risk factor analysis revealed young age, day care attendance, and number of antibiotic courses to most reliably predict the presence of resistant microorganisms.

KEY WORDS — antibiotic resistance, *Hemophilus influenzae*, *Moraxella catarrhalis*, otitis media, *Streptococcus pneumoniae*.

INTRODUCTION

Over the past 50 years, physicians and the pharmaceutical industry have waged war on otitis media (OM) with antimicrobial therapy. Recently, scientists and the media have expressed concern about the emergence of drug-resistant strains of microorganisms causing OM. Antimicrobial prescribing practices aimed at treating pediatric infections have been implicated in the development of drug-resistant OM.[1-3] Specifically, the indiscriminate treatment of upper respiratory tract infections and asymptomatic middle ear effusions with broad-spectrum antibiotics without an obvious bacterial source has been condemned.[1,3] In addition, the wisdom regarding the long-term use of prophylactic antibiotics to prevent recurrent acute OM has been questioned, as this seems to place evolutionary pressure on the development of resistant strains of bacteria.[4]

Streptococcus pneumoniae is the major etiologic pathogen in OM. This organism develops resistance via genomic alterations resulting in changes in cell membrane structures called penicillin-binding proteins. The penicillin-binding proteins are enzymes responsible for the synthesis of peptidoglycans used for bacterial cell wall formation.[5] The result is a decreased binding affinity for penicillin, as well as other antibiotics. In contrast, *Moraxella catarrhalis* and *Haemophilus influenzae* develop resistance by producing β-lactamase enzymes. Although β-lactamase–resistant drugs can be utilized with *H influenzae* and *M catarrhalis*, the addition of lactamase inhibitors does not help in restoring antibiotic effectiveness with pneumococcal resistance. Consequently, the utilization of β-lactamase–resistant antibiotics, including extended-spectrum cephalosporins or broad-spectrum penicillins plus lactamase inhibitors (clavulanate or sulbactam), in the treatment of pneumococcal resistant infections seems to offer no advantage.[5]

For many years, *S pneumoniae* was considered to be exquisitely sensitive to penicillin. However, the incidence of resistance of this organism to penicillin and other antibiotics has been increasing worldwide at a rapid pace over the past decade. From 1987 to 1992, the overall proportion of antibiotic-resistant *S pneumoniae* strains highly resistant to penicillin increased more than 60-fold (from 0.02% to 1.3%).[4] In day care center studies from various locations in the United States, the prevalence of nasopharyngeal cultures positive for antibiotic-resistant *S pneumoniae* has ranged from 11% to 61%.[4,6-9] Resistant strains of *H influenzae* in middle ear fluid have been reported as frequently as 15% to 30% for both type B and nontypeable strains.[9] *Moraxella catarrhalis* in middle ear fluid has been shown in several studies to be resistant in 85% to 100% of isolates. These resistant organisms have been linked in various stud-

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From the Department of Otolaryngology–Head and Neck Surgery, Eastern Virginia Medical School, Norfolk, Virginia. Funding provided through EVMS Institutional Research Grant. Administrative costs and statistical analysis provided by the Department of Otolaryngology–Head and Neck Surgery. Tym-tap provided by the Xomed-Treace Company.

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ies to an increased use of prophylactic antibiotics, the introduction of newer cephalosporins, and utilization of day care facilities.[4,6-8]

In this study, we sought to determine the prevalence of resistant *S pneumoniae* and other pathogenic microorganisms isolated from middle ear fluid of children undergoing placement of ventilation tubes. Further, the extent of their resistance to common classes of antibiotics and the risk factors associated with this resistance were examined.

MATERIALS AND METHODS

All children with middle ear effusions undergoing tympanostomy tube insertion at the Children's Hospital of the King's Daughters (CHKD) by attending physicians in the Department of Otolaryngology–Head and Neck Surgery at Eastern Virginia Medical School (EVMS) were eligible for this study. At the preoperative office visit, informed consent was obtained, and parents and caregivers were then asked detailed questions regarding the child's exposure to day care and secondary smoke, breast-feeding, family history of ear disease, use of antibiotics (both prophylactic and therapeutic) in the prior 12 months, prior history of ear disease (especially prior tubes), and number of middle ear infections in the prior 12 months.

At the time of surgery, each ear with fluid of any type was included for study purposes. The fluid was characterized as serous, mucoid, or purulent. The fluid characterization was based on the predominant fluid type. Cultures of the effusions were obtained in the following manner: the external auditory canal was sterilized with Betadine solution by infusing Betadine into the canal and allowing the solution to remain in place for approximately 60 seconds. This duration was chosen based upon the initial culture contamination results. The Betadine solution was then aspirated in its entirety, and the external canal was irrigated copiously with normal saline until clearing. Myringotomy was performed in the anterior inferior quadrant of the tympanic membrane. Middle ear fluid aspirates were then collected in a sterile Tym-tap (Xomed-Treace). Specimens were immediately plated onto sheep blood agar and chocolate agar plates in the operating room and then placed in 5% carbon dioxide bags. The cultures were then sent on ice to the CHKD/EVMS Center for Pediatric Research for identification and analysis.

After the isolates were identified, *S pneumoniae* cultures were tested for oxacillin resistance. Each specimen underwent minimum inhibitory concentration (MIC) testing by utilizing the Epsilometric test (E-test). The E-test is performed with a plastic strip containing concentration gradients for selected antimicrobial agents.[5] Resistance levels were determined from MIC values set by the 1995 National Committee for Clinical Laboratory Standards. The MIC levels were determined for multiple antibiotics, including penicillin, clindamycin, erythromycin, cefotaxime, and trimethoprim-sulfamethoxazole. All *H influenzae* and *M catarrhalis* isolates were tested for β-lactamase production.

The serotypes of *S pneumoniae* were determined through capsular reaction tests on pooled antisera. The specific antiserum utilized was Pneumotest, which is based on 12 pooled antisera that allow typing of 90% to 95% of pneumococci.[10] After identification of *S pneumoniae*, a bacterial suspension was made by mixing bacterial colonies with normal saline. The tests were performed by mixing an aliquot of bacterial suspension with a loopful of antiserum, diluted 1:8, on a microscope slide. The slide was then observed under phase contrast 100× magnification with a positive reaction showing agglutination with the capsules becoming more visible. Type-specific antisera were used to resolve discrepancies.

Statistical analysis was then performed utilizing polychotomous logistic regression with the ear as the unit of analysis. Positive cultures with susceptibility, positive cultures with resistance (intermediate or highly resistant), and negative cultures were the outcome variables. Detailed statistical analysis was performed under the direction of the Department of Biostatistics at EVMS.

RESULTS

There were 297 patients cultured, with a total of 414 ears undergoing aspiration. Sufficient data were available to include 244 subjects (total population) and 355 ears in the statistical analysis. Serotyping was performed on all cultures positive for *S pneumoniae*, including those without sufficient data for a complete risk factor analysis.

Demographic and risk factor analyses and comparisons are listed in Tables 1 and 2. The indications for placement of ventilation tubes were persistent middle ear fluid causing hearing loss (42.8%) and recurrent acute OM (57.2%). Positive cultures were obtained from 105 of 355 (29.6%) ears cultured. A positive culture breakdown by culture result showed 9.6% *S pneumoniae*, 12.1% *H influenzae*, 5.6% *M catarrhalis*, and 2.3% nonpathogenic. Nonpathogenic organisms included *Pseudomonas*, *Staphylococcus*, and *Neisseria* species for a total of 8 specimens. *Haemophilus influenzae* was the predominant organism isolated, with 40.9% of isolates with positive cultures harboring this organism. Negative cultures were

TABLE 1. DEMOGRAPHIC COMPARISON OF CULTURE RESULTS

Demographic	*Total Population*	*Positive Cultures*	*Negative Cultures*
Subjects	244	86 (35.2%)	158 (64.8%)
Ears	355	105 (29.6%)	250 (70.4%)
Sex			
Male	62.0%	63.9%	60.1%
Female	38.0%	36.1%	39.9%
Race			
White	75.0%	79.4%	72.5%
Black	22.0%	19.6%	24.8%
Other	3.0%	0.0%	2.7%

Cultures are reported by ear.

TABLE 2. RISK FACTOR COMPARISON OF CULTURE RESULTS

Demographic	*Total Population*	*Positive Cultures*	*Negative Cultures*
Mean age (mo)	40.7	29.6	44.1
Mean No. of infections	5.4	5.6	5.3
Mean No. of antibiotics	4.7	5.2	4.7
Day care	50.8%	61.9%	52.7%
Smoking	26.6%	16.5%	28.3%
Breast-feeding	15.2%	26.8%	10.9%
Prior tube	12.3%	10.3%	12.8%
Family history	39.3%	45.4%	37.6%
Prophylactic antibiotics	15.6%	15.5%	16.3%

Cultures are reported by ear.

obtained in 250 of 355 (70.4%) ears. A breakdown of culture results can be seen in Figs 1-3.

Examination of resistance to the tested antibiotics showed that 20 patients with *S pneumoniae* cultures had either highly resistant or intermediately resistant strains. Nearly equal numbers of *M catarrhalis* (20) and *H influenzae* (19) isolates were β-lactamase–producing. This finding indicates that nearly 25% of all patients with middle ear fluid were harboring a resistant strain of bacterium. Of all ears tested, 20% had a resistant organism (*S pneumoniae* 7.3%, *H influenzae* 7.9%, and *M catarrhalis* 5.6%). The susceptibility profile demonstrated that 76.5% of isolates positive for *S pneumoniae* were resistant to the 5 antibiotics tested (Fig 4). β-Lactamase production was present in 100% of *M catarrhalis* and 70% of *H influenzae* isolates (Fig 5).

Statistical analysis revealed younger age to be predictive for resistant strains of *S pneumoniae* ($p < .0001$) and for resistant organisms in general ($p = .0006$). There was a direct relationship between decreasing age and the outcome of a resistant organism. After adjustment for age, no other risk factors were statistically significant. When all 3 organisms were studied, exposure to a day care environment with 3 or more children was significantly related to outcome ($p = .04$). The number of courses of antibiotics, race, prior history of tubes, family history of ear disease, and exposure to prophylactic antibiotics did not correlate with the presence of resistant organisms. There was no apparent beneficial effect from avoiding secondary cigarette smoke or from breast-feeding.

Serotyping analysis revealed 6, 9, 14, 19, and 23 to be the most prevalent subtypes of *S pneumoniae*. The percentages of intermediate and highly resistant strains by serotype were 86%, 100%, 50%, 75%, and 75%, respectively.

DISCUSSION

An analysis of risk factors implicated in the development of acute and chronic OM has been accomplished in several studies. Factors associated with an increased risk of developing OM include male sex, race, younger age, exposure to cigarette smoke, atopy, day care, use of pacifiers, season, and low socioeconomic status.[7,8,11-23] Infante-Rivard and Fernandez[21] discuss several of these risk factors in detail. Few studies to date have been conducted to determine risk factors associated with the development of multi-drug-resistant strains.[7]

Streptococcus pneumoniae penicillin resistance was first reported in 1967, by Hansman and Bullen[24] in Australia. In the 1970s to 1980s, the incidence of

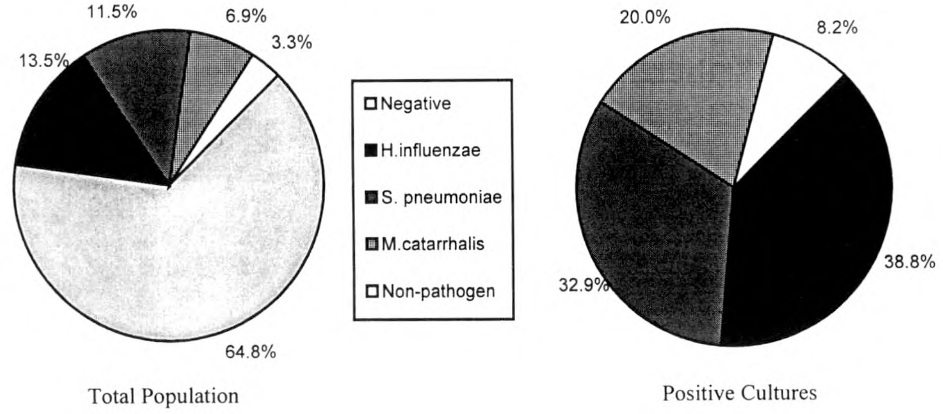
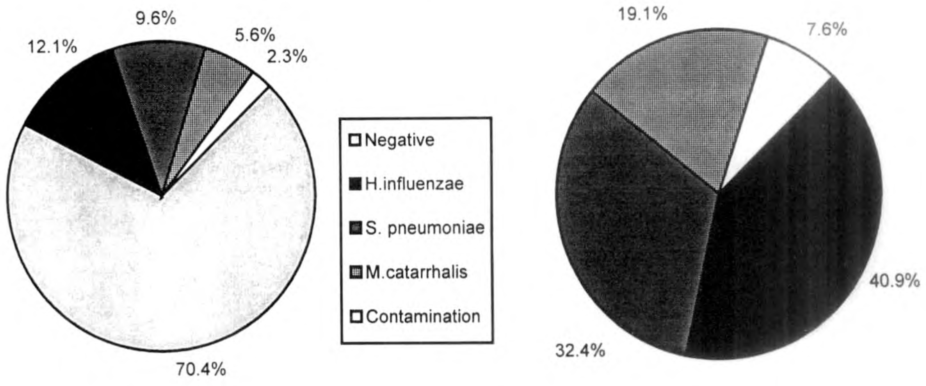


Fig 1. Microorganism isolates by individual patient, expressed in percentages of total population and of positive cultures.



Total Population

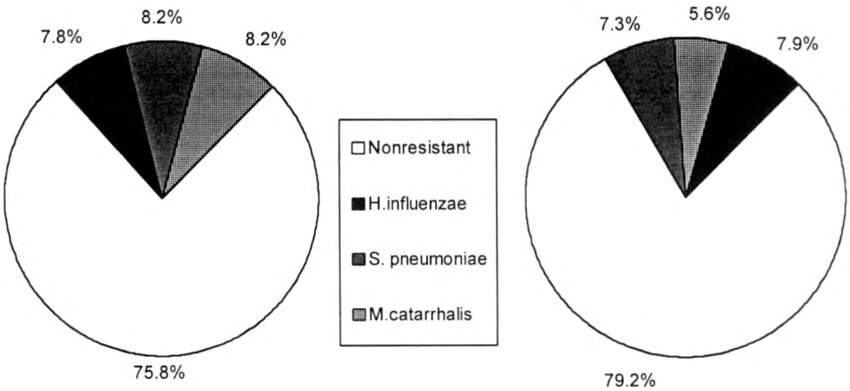
Positive Cultures

Fig 2. Microorganism isolates by individual ear, expressed in percentages of total population and of positive cultures.

resistance increased dramatically outside the United States. This was documented primarily in reports from Spain, Brazil, and South Africa; in these countries, antibiotics are available as over-the-counter preparations. Since the early 1980s, the US Center for Disease Control and Prevention has been tracking the increasing frequency of multi-drug-resistant isolates of *S pneumoniae*.[6]

In the present study, the racial and demographic breakdown of the population undergoing ventilation tube insertion compares well with that in other studies in the literature. The relatively high percentage of patients in our study undergoing placement of ventilation tubes for persistent middle ear effusions (42.7%) may explain why the mean number of infections (5.4) and courses of antibiotics (4.7) in the prior 12 months is low. Our finding that the number of courses of antibiotics did not correlate with culture outcome can also be explained by this phenomenon. Although the number of antibiotic courses in the prior 12 months did not quite reach a statistically significant level with respect to the presence of resistant organisms (p = .06), an association did seem to be present. This association between antibiotic utilization and development of resistant strains has been previously demonstrated in several studies. Ford et al[23] compared children with chronic OM with resistant strains of *S pneumoniae* to those without resistant strains and found that the only significant differences were that the children with resistant strains had received more β-lactamase antibiotics within 3 months of culture and that a significant number of these had received amoxicillin–clavulanic acid. Reichler et al[25] demonstrated that prophylactic antibiotics and frequent use of antibiotics carried a significant increased risk of a patient's becoming a nasopharyngeal carrier of a resistant strain of *S pneumoniae*.

In our study, young age and day care exposure clearly show an increased risk for the development of resistant strains of microorganisms in middle ear fluid at the time of ventilation tube placement. Younger age was implicated as a risk factor for development of both multi-drug-resistant *S pneumoniae* and β-lactamase–producing *H influenzae* and *M catarrhalis*. The data indicate that children 9 months or younger carried a 24% chance of harboring multi-drug-resistant *S pneumoniae*. Day care exposure was defined as exposure for at least 1 day of the week to 3 or more unrelated children. Our analysis revealed that children in a day care situation, as defined above, were twice as likely to develop a resistant organism



By Patient

By Ear

Fig 3. Microorganism isolates with resistant cultures, listed by patient and by individual ear.

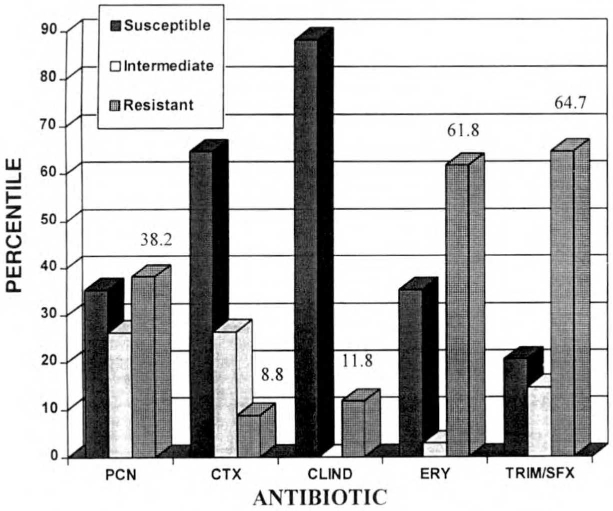


Fig 4. Susceptibility profile for *Streptococcus pneumoniae* isolates. Percentages of resistant strains to each antibiotic are shown. PCN — penicillin, CTX — cefotaxime, CLIND — clindamycin, ERY — erythromycin, TRIM/SFX — trimethoprim-sulfamethoxazole.

as those children in home care environments. Block et al[7] demonstrated by univariate analysis that day care attendance and younger age were statistically associated with the development of resistant strains. Reichler et al[25] clearly elucidated a relationship between day care exposure and nasopharyngeal cultures of a resistant strain of *S pneumoniae* (type 23F). This particular strain was isolated from the middle ear fluid of a child attending a day care center in Ohio and was also found in nasopharyngeal cultures from the index day care center. However, no cultures were resistant in 2 other centers in the same area. Direct transference of resistant microorganisms is implicated by this finding. Infante-Rivard and Fernandez[21] discuss several other publications that show a significant relationship between acute OM and day care attendance. However, direct transmission of bacterial pathogens has not been distinctly proven. Day care exposure has been shown to be a risk factor for the occurrence of viral upper respiratory tract infections.[26] This exposure to viral pathogens often leads to acute OM.[11] Therefore, in our study it may be the increased exposure to viral infections in a day care environment that predisposes these children to OM with subsequent increased antibiotic utilization and, thus, the development of resistant organisms.

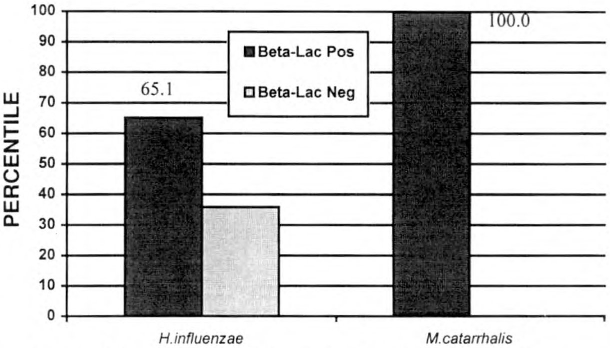


Fig 5. β-Lactamase production of *Haemophilus influenzae* and *Moraxella catarrhalis* isolates. Values represent percentages of cultures that produce β-lactamase.

Antibiotic susceptibility profiles demonstrate that the resistance in our locality is increasing, with nearly 40% of *S pneumoniae* isolates being resistant to penicillin. This represents a considerable increase over the incidences reported from Kentucky middle ear fluid isolates (28%) and Tennessee nasopharyngeal isolates (29%) several years ago.[4] The susceptibility profile also indicated that three quarters of the isolates were intermediately resistant or resistant to the antibiotics tested. The antibiotic profile chosen very closely approximates that utilized by the pediatricians in our community. Further, β-lactamase production for *H influenzae* in these chronic OM ears is dramatically higher than that in previous reports for nontypeable strains in the United States.[8] The universality of *M catarrhalis* β-lactamase production we demonstrated is consistent with that found in earlier studies.[27]

The predominant serotypes of *S pneumoniae* isolated in this study were among the most commonly reported in the literature. However, taken together, types 6, 9, 14, 19, and 23 were found in higher percentages among resistant cultures than were documented in prior reports.[8,20]

CONCLUSION

Drug-resistant microorganisms in the middle ear fluid of children undergoing ventilation tube insertion are increasing in incidence. This finding is highly correlated with day care attendance by children of young age and is also associated with the frequent use of broad-spectrum antibiotics. A multi-drug-resistant strain should be suspected in any child less than 12 months of age with persistent middle ear fluid or with recurrent bouts of acute OM who also attends a day care center with 3 or more children. In such cases, early intervention with either tympanocentesis with culture or ventilation tube insertion with culture should be considered.

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EXTRANEOUS ROUND WINDOW MEMBRANES AND PLUGS: POSSIBLE EFFECT ON INTRATYMPANIC THERAPY

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Recently there has been increasing interest in the possibility of treating inner ear disorders by application of medication into the middle ear on the premise that it will diffuse through the round window membrane into the inner ear. We examined 202 temporal bones from 117 patients to determine the frequency of round window niche obstruction. Patients ranged in age at the time of death from 31 to 97 years. Eleven percent of the ears were found to have fibrous tissue or a fat plug, and 21% had an extraneous (false) round window membrane. Of the 85 patients from whom both temporal bones were examined, 56% had no obstruction in either ear, while 22% had obstruction in both ears. We conclude that anatomic variations of the round window niche may explain the wide variations found in dosage of medication required to produce a clinical result.

KEY WORDS — fat plug, fibrous tissue, inner ear, intratympanic therapy, medication, middle ear, round window membrane, tinnitus, vertigo.

INTRODUCTION

There has been interest in treatment of inner ear problems such as vertigo and tinnitus by instillation of medication into the middle ear.[1-3] Substances have been shown to pass from the middle to the inner ear via the round window membrane (RWM),[4] which, in the human, is located deep in a recess between the middle ear and inner ear (Fig 1).

Titration therapy has been found to be necessary to reach the desirable therapeutic result.[3] Reasons for the variability in required drug level include the presence of certain factors that affect the passage of the substances from the middle ear through the RWM. One of these factors is molecular size.[4] If the size is less than 1,000 molecular weight, as in different types of antibiotics (gentamicin, streptomycin, neomycin, etc) and steroids, the substances are transmitted actively within a short time,[4] while substances with molecular weight of over 1,000 (eg, endotoxins, human albumin, firritin) are transported by pinocytosis.[4,5] Other factors include molecular configuration, concentration, liposolubility, and electrical charge. In addition, the thickness of the RWM[6] or the presence of an extraneous (false) RWM, which has been called a veil,[7] may affect movement of substances through the membrane. Otoendoscopy has been used to remove the extraneous RWM, thus making the RWM more accessible to the local application of medication.[1,2]

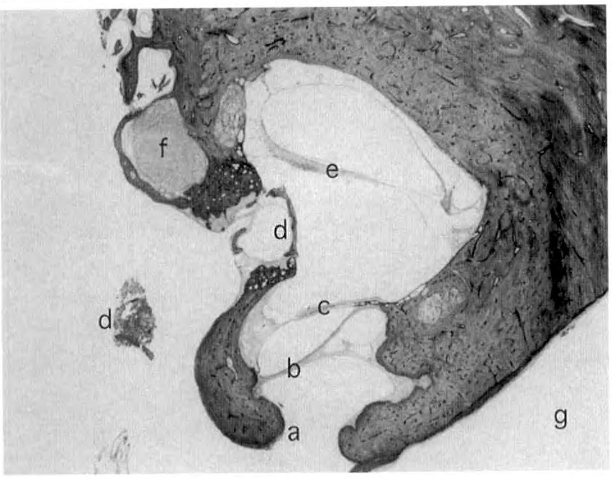

Fig 1. Vertical section of temporal bone showing operculum (a), round window membrane (RWM; b), osseous spiral lamina (c), stapes capitulum and footplate fixed by otosclerosis (d), utricle (e), facial nerve (f), and jugular bulb (g) (H & E, original ×20).

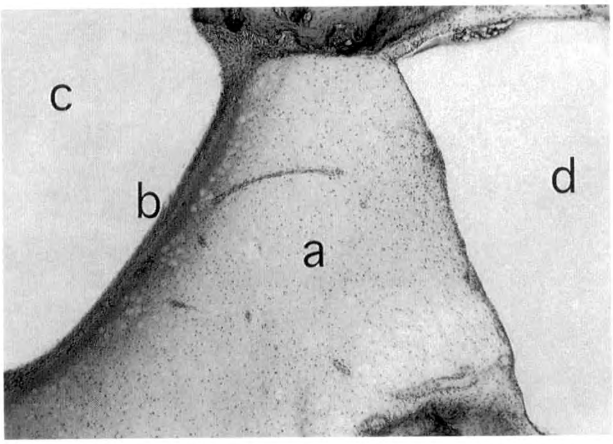

Fig 2. Round window niche with fibrous plug (a), RWM (b), scala tympani (c), and middle ear cavity (d) (H & E, original ×100).

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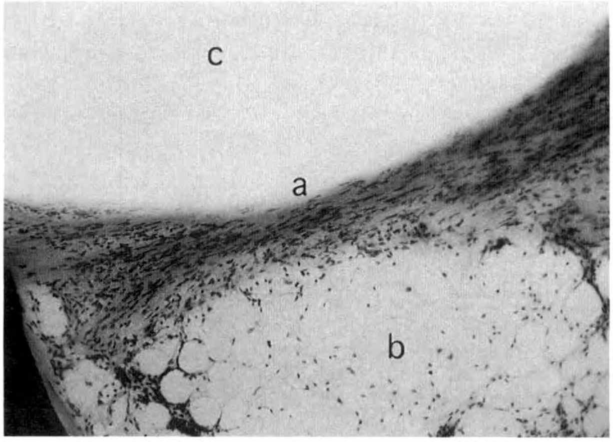

Fig 3. RWM (a) obstructed by fat plug (b) (H & E, original ×120). c — scala tympani.

We have found the round window niche may also be obstructed by a tissue plug, which could play a role in the difficulty of passage of certain substances via the RWM. These plugs can be of 2 types: fibrous (Fig 2) or fatty (Fig 3). This study was designed to determine the prevalence of fibrous and fatty plugs, as well as extraneous membranes (Fig 4).

MATERIALS AND METHODS

We examined 117 sequential pairs of temporal bones prepared in our laboratory from 1972 to 1982 from patients who had pledged them for postmortem study. The bones had been removed intracranially with the block technique, fixed in buffered formalin, decalcified in ethylenediaminetetraacetic acid, embedded in celloidin, and then cut into 20-µm sections. Every 10th section was mounted and stained with hematoxylin and eosin; the remaining 9 of each 10 sections were stored on numbered tissues in 80% alcohol. Thirty-four ears were discarded because the RWM was not present in the slides. There were 104 right ears and 98 left ears that were suitable for evaluation, with both ears included in 85 cases. The number of slides encompassing the round window varied among bones, from 6 to 10, with a mean of 8 slides; thus, we were confident that the niche was completely obstructed in all cases reported. Female patients outnumbered male patients 2 to 1. The age at time of death ranged from 31 to 97 years.

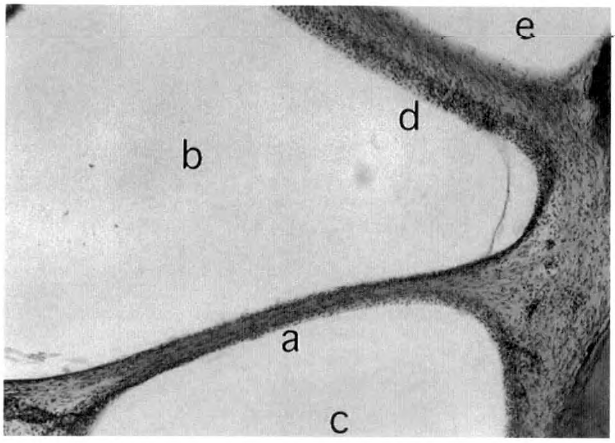

Fig 4. Extraneous RWM (a) obstructing round window niche (b) (H & E, original ×100). c — middle ear cavity; d — round window membrane; e — scala tympani.

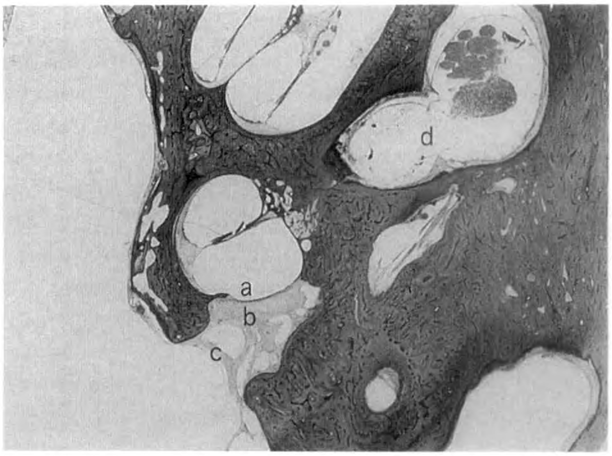

Fig 5. Scala tympani at RWM (a) (H & E, original ×20). Round window niche is blocked by fibrous plug (b) and extraneous RWM (c). d — internal auditory canal.

RESULTS

Sixty-seven of the ears were found to have obstruction of the round window niche. There were 3 fat plugs in the 202 specimens (1.5%), 21 fibrous plugs (10.4%), and 43 extraneous RWMs (21.3%; Fig 5), for an overall prevalence of 33.2% (95% confidence interval, 26.3% to 40.1%). The rate did not differ when comparing cases with only 1 temporal bone (34.4%) to cases in which both ears were included (32.9%). In bilateral cases, the 2 ears frequently had different obstructions (see Table). For the 85 patients in whom both temporal bones were included, 48 (56.5%) had no obstruction in either ear, while 19 (22.4%) had obstruction in both ears. The remaining 18 (21.1%) had an obstruction in 1 ear but not the other. Seven ears were found to have signs of chronic inactive otitis media; 1 of them had a fibrous plug and 3 had extraneous RWMs, while 3 of the 7 had normal round window findings.

FINDINGS IN 85 PATIENTS IN WHOM BOTH TEMPORAL BONES WERE EXAMINED

	Right Ear				
Left Ear	*Fibrous Tissue*	*Fat*	*Membrane*	*Nothing*	*Total*
Fibrous tissue	4	1	1	5	11
Fat		1	1		2
Membrane	2		9	3	14
Nothing	1		9	48	58
Total	7	2	20	56	85

DISCUSSION

One reason for the individual variation in the amount of intratympanic drug required to achieve therapeutic inner ear results becomes evident on examination of a number of temporal bones. The human RWM is located almost horizontally deep in a niche and partially separated from the middle ear operculum (Fig 1). The passage of medications to the membrane can be hindered by the presence of extraneous membranes stretched across the opening from the middle ear to the round window[6] or by fibrous or fat tissue plugs obturating the niche. These obstructions occur singly or in combination in 33% of temporal bones.

The epithelium covering the middle ear side of the RWM is a significant barrier to the passage of substances through the membrane and into the inner ear because of its tight junctions. The transportation through the epithelium must be by pinocytosis.[7] Passage through the fibrous layer and the inner epithelial lining is facilitated by the lack of tight junctions. The extraneous membranes are composed of 2 sheets of tight-junction epithelium and a fibrous layer, thus tripling the tight-junction epithelial barriers to the passage of drugs into the inner ear. The plugs that fill the round window niche may be as thick as 1 mm, in contrast to the normal RWM, which is 0.0638 to 0.677 mm in thickness.[8] The fat plugs may be of the same dimensions as the fibrous plugs and may be particularly resistant to the passage of fat-insoluble substances.[9] The fat cells are probably derived from osteoprogenitor cells that can differentiate into adipose cells.[10]

A standard dosage of medications used in the middle ear to produce a therapeutic result in the inner ear cannot be established in the presence of the extraneous membranes and round window plugs that occur in 33% of ears. To overcome this problem, Silverstein et al[1,2] have recommended examining the round window niche with endoscopes and using any necessary surgical procedures to minimize the obstruction to the passage of the drugs to the inner ear. Unless the round window niche is cleared of obstructions, continuous titration over an indeterminate time will continue to be necessary to achieve therapeutic results and avoid overdose complications.

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ESTIMATION OF VOLUME REFERENT BONE TURNOVER IN THE OTIC CAPSULE AFTER SEQUENTIAL POINT LABELING

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Using fluorochrome labeling and a newly validated method for bone turnover estimation, we determined absolute values for canine perilabyrinthine bone remodeling. The overall capsular bone turnover was found to be 2.1% per year, compared to 13.9% per year for the neighboring cranial bones and 7.4% per year for the humerus compacta. This gross 2.1% per year conceals a vast range, from 0.13% per year for the innermost perilymphatic zone, through a centrifugal increment toward 8% to 10% per year in the periphery. The underlying individual bone remodeling units exhibit a similar centrifugal pattern in numerical density and size. These findings indicate an inhibition of remodeling, seemingly emanating from the perilymphatic spaces, and affecting both the activation of osteoclasts and the extent of resorption by the osteoclasts. These values satisfactorily explain the preservation of such fetal remnants as the globuli ossei, the interglobular spaces, and the skein bone. In humans, local ineffective inhibition of bone resorption may play a role in the initiation of otosclerosis.

KEY WORDS — endochondral layer, histomorphometry, stereology, undecalcified histology.

INTRODUCTION

As early as at the turn of the last century, otologists were aware of the unique bony development of the otic capsule, characterized by a late onset of endochondral ossification in the 16th week of gestation, ending within 8 weeks. At this time, the spaces of the labyrinth have acquired their final adult size. Substantial amounts of nonexchanged cartilage matrix persist, and the original anlage does not grow, apart from the continuous bony apposition to the outer surface of the otic capsule in later life and the cancellous compaction of the marrow cavities that fades out around 2 years postnatally.[1,2]

A triad of features of the middle endochondral layer of the otic capsule attests to an inhibition of postnatal capsular bone remodeling. First, the calcified cartilage islands, the so-called interglobular spaces, may be seen all through life. Second, a fracture of the temporal bone rarely heals with callus formation and subsequent bony remodeling in the portion of the fracture traversing the petrous bone.[3,4] Finally, it has been shown, by the use of bone-seeking fluorochromes, that label uptake is gradually reduced approaching the perilymphatic space (PLS), with practically full inhibition of bone exchange in a zone of 500 μm from the PLS.[5,6] Overall capsular bone remodeling rates appear to be low, and individual bone remodeling units (BRUs) do not follow a straight course through the perilabyrinthine bone. Labeled BRUs generally appear short and tortuous as compared to BRUs of extracapsular compact bone. These features render estimation of bone remodeling by the use of well-known histomorphometric methods inapplicable to the petrous bone, because the steric preconditions required are not met here.[7,8] At present, no absolute values exist on capsular bone turnover (BTO).

Recently, a new method to estimate BTO in situations with very low BTO and irregular, multidirectional BRUs, as is the situation in the otic capsule, was developed and tested against classic methods in canine long bones.[7] The method is based on fluorochrome labeling and measurements of the fraction that the interlabel area (ILA; the area between labels) makes up of the total bone area. This paper presents its application to perilabyrinthine undecalcified canine temporal bone specimens labeled with a sequence of osteofluorochromes. It provides an estimate of capsular BTO, for the first time in absolute values.

MATERIALS AND METHODS

Eight male mongrel dogs, 16 months old, were kept under standard conditions for 6 months. They had a weight-bearing implant inserted into the medial fem-

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From the Department of Otolaryngology–Head and Neck Surgery, University of Copenhagen, Copenhagen (Frisch, Sørensen, Bretlau), and the Biomechanics Laboratory, Aarhus University Hospital, Aarhus (Overgaard), Denmark. This study received economic support from The John and Birthe Meyer Foundation and The Gerda and Aage Haensch Foundation.

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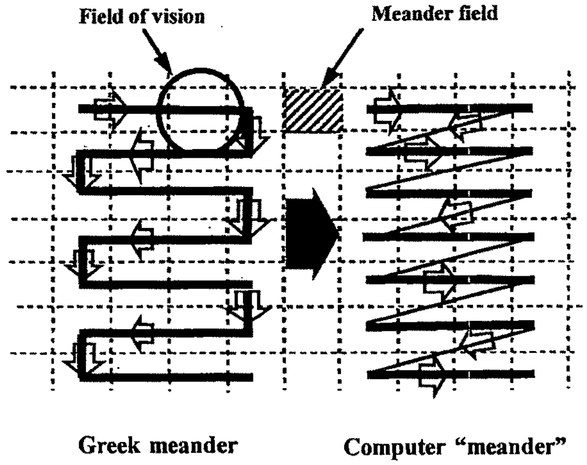

Fig 1. Meander sampling principle: classic Greek pattern (left) and computer-assisted procedure (right). Latter divides delineated area into number of user-defined rectangles (truncated) that subsequently are examined stepwise on monitor, from left to right. Circle indicates microscopic field of vision in every step.

oral condyle bilaterally for an independent orthopedic study, approved by the national board of animal experiments.[9] All animals were subjected to a scheme of sequential point labeling with single intravenous injections of calcein green 15 mg/kg (Sigma, St Louis, Mo) at days 0 and 13 and subsequent intravenous injections of tetracycline hydrochloride 20 mg/kg (Dumex, Denmark) at days 29 and 39. All animals were anesthetized with pentobarbital sodium and painlessly killed with potassium chloride at day 43.

The temporal bones were removed with the Schuknecht temporal trephine (Stryker, Kalamazoo, Mich)[10] immediately after sacrifice and immersed in 10% ethanol. After 1 week, the specimens were trimmed, dehydrated in 63% and 99% ethanol, and defatted twice in ether/acetone 1/1. Following infiltration with pure methyl methacrylate (MMA; BDH, United Kingdom), the specimens were embedded in MMA, primed with 0.5% benzoyl peroxide (Fluka Chemie AG, Switzerland), softened with 0.5% dibutyl phthalate (Merck, Germany), and polymerized at 32°C.

Transverse sections of the right temporal bones were produced on an Accutom-2 milling machine (Struers, Denmark), reduced to a final thickness of 30 µm by wet grinding on a Knuth-Rotor grinding machine (Struers), and mounted unstained.

Measurements of areas and distances were performed with the CAST-Grid system (Olympus, Denmark). The CAST-Grid system is a semiautomatic computer-assisted device for microscopic stereological estimation. On the monitor, the video-transferred digitized image of the histologic slice can be overlaid (superimposed) by customizable point grids, by which the area of any 2-dimensional structure can be estimated by counting the number of grid points overlying that particular structure, because each point represents a known area.[8] By means of clicking and dragging the computer mouse, lengths can be measured directly. Any area of interest may be outlined on-screen by the cursor at low-power magnification. Then this area can be scanned at higher magnifications by computer-controlled movements of the motorized object table, in the so-called meander pattern, covering the whole delineated area (Fig 1). This function ensures that any structure is seen once and only once. With this technique, the areas of capsular bone (BAC) and neighboring extracapsular bone (BAXC) were estimated at low-power magnification.

In addition, for dog 1, considered representative of all, estimates of bone areas within concentric 400-µm-wide perilabyrinthine zones were made (standard area distribution).This was done by simple on-screen grid overlay, measuring the distance for every point to the nearest PLS in that plane.

The fluorescence microscopic procedures were undertaken at optic magnification ×100 in 2 steps. First, with the meander sampling function, the whole slice was run through, yielding a quick estimate of the extracapsular ILAs of secondary osteons (ILAXC). Then every labeled capsular BRU was detected, a finer grid was arbitrarily overlaid, and individual ILAs were obtained (ILAC). Their individual distances to the closest PLS could simultaneously be measured. It has been proposed to divide BRUs into zonal (or abortive) and regular (or normal) BRUs, respectively having their shortest diameter below or above 80 µm.[11,12] In 7 dogs the shortest BRU diameter was noted.

The ratio of ILA to total bone area (ILAC/BAC in percent and ILAXC/BAXC in percent) was converted to yearly formative BTO through the following formulas[7]: Capsular bone turnover BTOC = (ILAC/BAC) × (365/LP) × 100% per year, and Extracapsular bone turnover BTOXC = (ILAXC/BAXC) × (365/LP) × 100% per year, where LP is the labeling period in days.

It is important to realize that these surface-derived percentages are fully convertible to volume referent percentages, with no further requirements to the data, due to the Cavalieri principle.[8] By using the standard area distribution, the observations within each of the 400-µm-wide perilabyrinthine intervals could be related to the relevant area fraction of that zone, and the findings in each of the concentric zones could be compared. A paired 2-tailed Student's t-test was used in the comparisons.

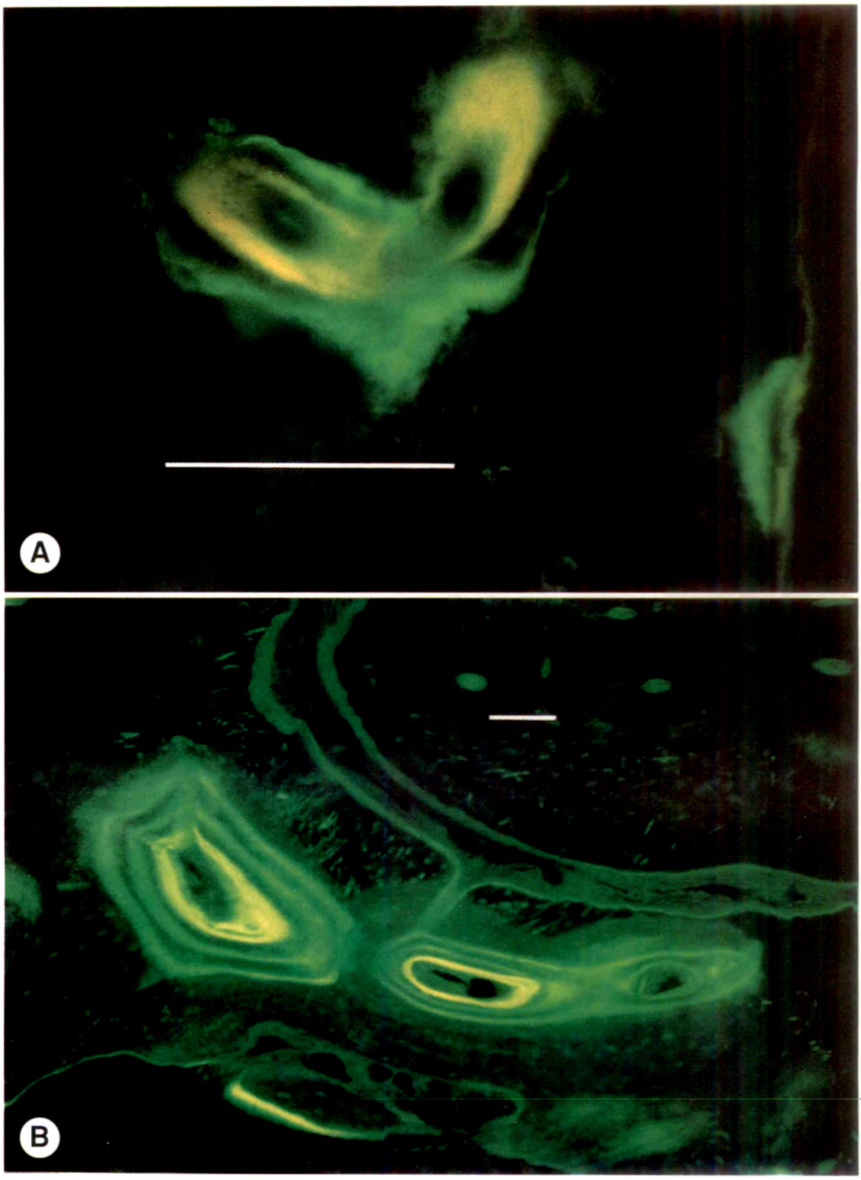

Fig 2. Photomicrographs of bone remodeling units (BRUs), labeled with calcein and tetracycline. A) Typical small, tortuous capsular BRU, 700 μm away from posterior semicircular canal (original ×400, bar — 10 μm). Streak of appositional bone is seen to right. B) Typical cross-sectioned extracapsular BRUs, just outside capsular suture in upper right corner (original ×100, bar — 100 μm). Note different magnifications.

RESULTS

Typical capsular and extracapsular BRUs are shown in Fig 2. The ILA of 1,894 labeled capsular BRUs was estimated, together with simultaneous measurements of diameter (1,632 BRUs; 7 dogs) and distance to the PLS (1,875 BRUs; some slices without PLS). The mean capsular BTO compiled for all dogs was 2.1% per year (SD 1.1% per year), while the adjacent extracapsular bones had a mean BTO of 13.9% per year (SD 8.3% per year; p = .004). In the same dogs, we previously found the mean BTO in the humerus compacta to be 7.4% per year (SD 6.0% per year; p = .35).[7] Based on the standard area distribution derived from 1 dog and the mean capsular BTO rates for the perilymphatic concentric intervals, capsular BTO was calculated for an inner (<400 μm) zone and an outer (>5,000 μm) zone (Fig 3). The BTO values for each of the 8 dogs are shown in Fig 4. It is seen that the overall capsular BTO, and the innermost BTO in particular, is very low compared to the extracapsular skull values (p < .005). A uniform pattern was found in all dogs.

For all dogs, the mean label area of regular BRUs (>80 μm) and zonal BRUs (≤80 μm) was calculated for each perilymphatic interval. For the zonal BRUs, a conspicuous centrifugal increase in mean surface area was seen until a distance of approximately 3,200 μm. A similar distribution was not evident for normal BRUs (Fig 5). A marked centripetal reduction in numerical density of both regular and zonal BRUs was observed, together with a clear reduction of the proportion of normal BRUs (Fig 6). Close to the PLS, almost no regular BRUs were seen. The BTO was calculated within each perilabyrinthine interval. The contributions of regular and zonal osteons are plotted separately in Fig 7. Figure 8 shows the theoreti-

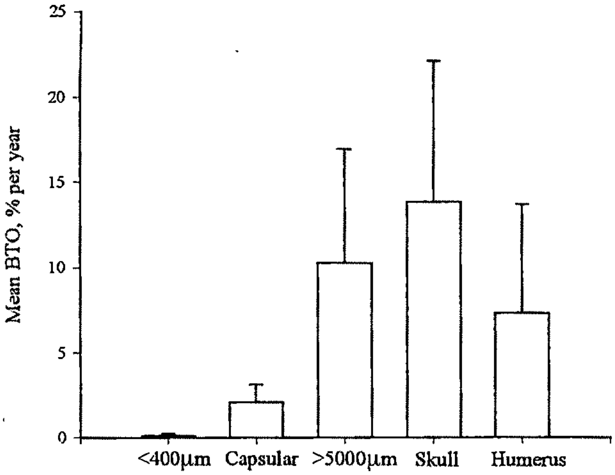

Fig 3. Comparison of mean bone turnover (BTO) for all 8 dogs, for capsular bone (total and 2 subdivisions: innermost 400-µm interval and all peripheral capsular bone >5,000 µm away from perilymphatic space [PLS]) and extracapsular bones (neighboring skull bones and humerus). Note very low bone turnover of innermost 400-µm interval of capsular bone adjacent to PLS. Bars — 1 SD.

cal removal of bone by remodeling, based on the BTO just found for each of the 400-µm intervals from the PLS, in the 2 situations in which the remodeling follows either a random, inversely logarithmic decay, or a highly selective removal pattern in which only old bone is remodeled away (see Discussion).

DISCUSSION

A quantification in absolute values of the BTO in the otic capsule is presented. A general discussion concerning the validity of the method for BTO estimation is held elsewhere,[7] leaving a few points of particular interest here.

Density. In theory, it would be preferable to measure the distances to the PLS for the labeled BRUs, as well as for the standard area distribution, not in the given plane, but in the space. As we measure now, we overestimate the proper distance in an unknown part of the measurements, since any point recorded may in fact be closer to a PLS just outside the section than to the one used as a referent within the section. A further disadvantage is that the top and bottom sections of the capsule are not available for measurements when they contain no recognizable PLS. However, for comparison between 2 parameters, both dependent on the same distance measurements such as the distributions of BRU label area and interval bone area, this uncertainty is canceled out.

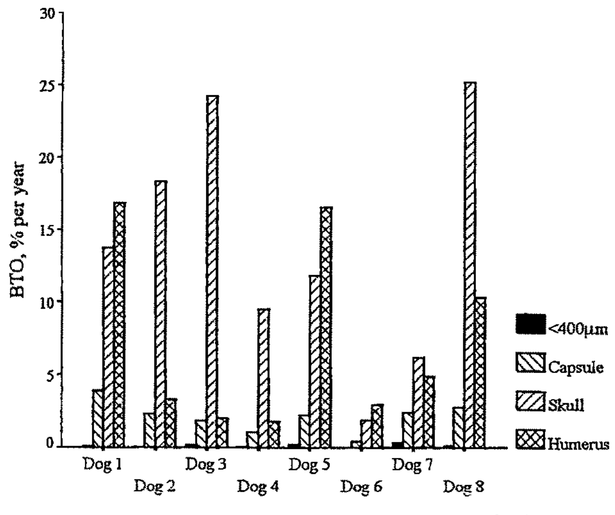

Fig 4. Individual dogs' capsular bone turnover for innermost 400 µm from PLS and overall capsular bone turnover compared to BTO for extracapsular adjacent skull bone and humerus.

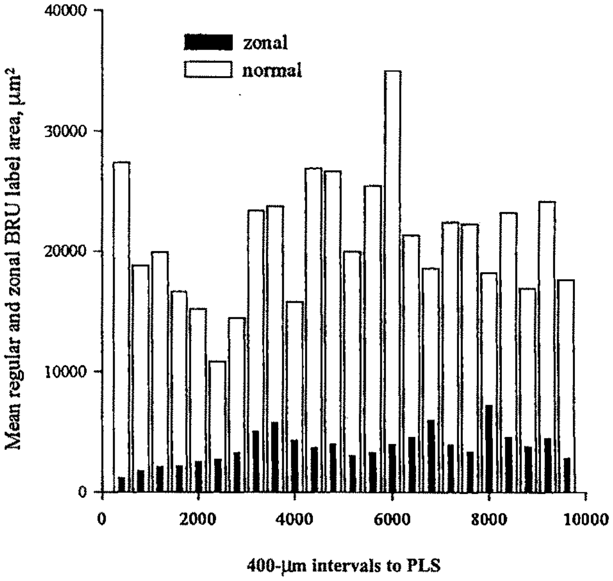

Fig 5. Distribution of mean label areas for regular and zonal (black bars) bone remodeling units (BRUs) within 400-µm intervals from closest PLS.

Establishing a standard area distribution on the ba-

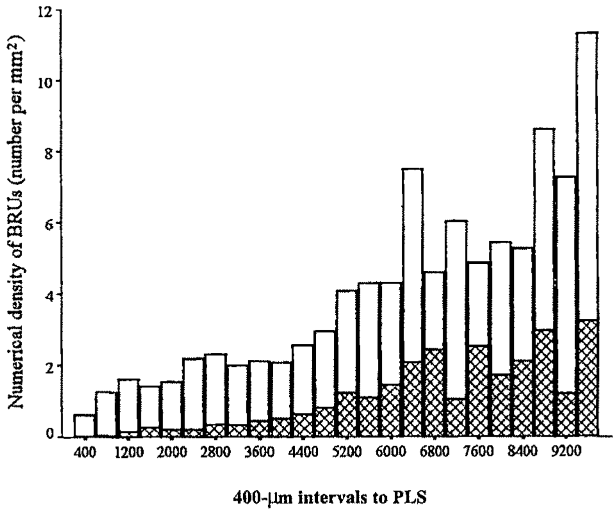

Fig 6. Numerical density distribution of regular (filled bars) and zonal (empty bars, stacked on top) BRUs within 400-µm intervals from closest PLS.

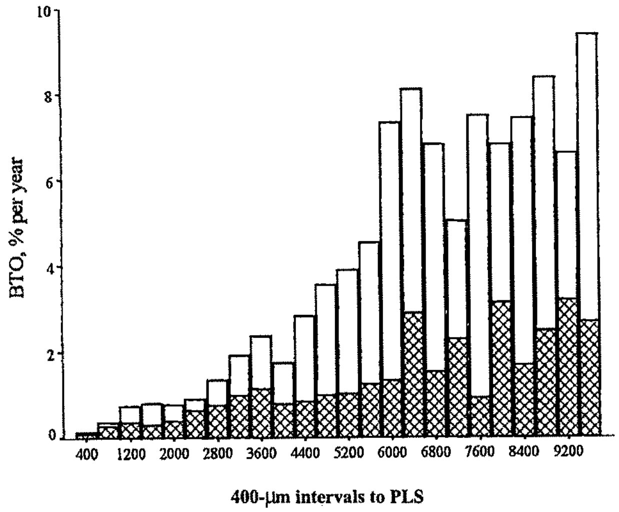

Fig 7. Yearly bone turnover due to regular (empty bars, stacked on top) and zonal (filled bars) BRUs within 400-µm intervals from closest PLS.

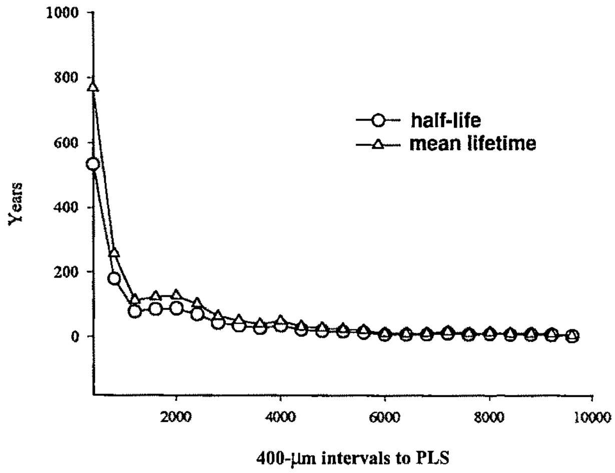

Fig 8. Theoretical mean lifetime and half-life of capsular bone removal by remodeling, related to 400-µm intervals from closest PLS (see Discussion).

sis of 1 single representative capsule is only an approximation to having individual area distributions for each animal. However, the plot for the BTO distribution for the dog providing the standard area distribution agrees with the overall findings (not shown).

Bone Turnover. The basic principle of BTO estimation is to establish a well-defined time window in the histologic slice. This is done by the 4 fluorochrome labels, and all bone seen between the first and last labels is the precise amount of bone made during the labeling period. Uncertainty may arise when not all labels are seen simultaneously. In cases with regularly spaced labels in larger osteons, it can be deduced from the topography of the labels which part of the recently appositioned bone was made in the labeling period.[7] In the otic capsule, the cement line (ie, the outer border of the BRU) is often vague and irregular, and very frequently, the labels are merging with no discernible distance, thus invalidating the sequence topography. Counting only the label area in which no ILA can be discerned will tend to underestimate the BTO — probably only slightly, though — whereas the inevitable blurring of the labels will tend to exaggerate it. None of these factors are considered as seriously influencing these results.

If bone remodeling is steady and random (ie, constant and without preference to bone of any particular age or quality), the exchange of bone will resemble the decay of radioisotopes with a half-life = $\ln 2/\lambda = \ln 2/\text{BTO}$. If, on the other hand, bone remodeling tends to remove older bone by preference (in analogy to the removal of older erythrocytes from the circulation), BTO is more accurately expressed by a mean lifetime = $\lambda^{-1} = \text{BTO}^{-1}$. The "more random" the bone exchange is, the more relevant the half-life, while the more specific the remodeling, the more relevant the mean lifetime. In long bones, remodeling does not appear totally random. There seems to be a preference toward the exchange of older or fatigue-damaged bone first.[13-15] This has not been shown for the otic capsule, and considering the unique inhibitory environment here, may not be valid. Within the closest 0 to 400 µm from the PLS, the mean BTO has been found to be 0.13% yearly. This is about 80 times less than the approximately 10% per year in the capsular periphery (Fig 3) and 100 times less than in the extracapsular cranial bones adjacent to the petrous bone. These bones have a higher content of marrow cavities, which gives them an appearance resembling cancellous bone. Cancellous bone is usually undergoing more remodeling than neighboring compacta.[12,16] However, even in comparison to proper compact bone with only a little porosity, such as the humerus cortex in the same animals, the inner capsular BTO was 57 times less[7] (Figs 3 and 4). With bone exchange of this magnitude, it will take 770 years to substitute all bone in the innermost interval with secondary osteons, if the exchange is so accurate that any bone volume is exchanged once and only once. This is hardly the case. With fully haphazard bone remodeling, the half-life for the exchange would be 530 years (Fig 8). If the values presented here are representative for all larger mammals with remodeling (and we do not have any human values, so far), any exchange pattern between these functional extremes may easily explain the presence of the skein bone and of the fetal calcified cartilage remnants (interglobular spaces) in senescence. Further, they explain satisfactorily such features of the otic capsule as no or slow healing of translabyrinthine temporal bone fractures and may contribute to the explanation of the presence of in vivo microfissures in human perilabyrinthine bone.

Bone Remodeling Units. Similar to the bone turnover pattern, a clear centrifugal increase in numerical density, mean label area, and diameter for the BRUs within a 400-µm interval indicates a centripetal inhibition. This is in agreement with former qualitative studies on human otic capsules exploiting accidental labeling with therapeutic tetracycline[17] and with findings in rabbits.[6] In the present study, practically all BRUs closer than 800 µm to the PLS are abortive, whereas the fraction of normal BRUs increases centrifugally. In long bones, zonal BRUs only contribute insignificantly to the total remodeling. Due to the very low overall remodeling in the otic capsule, with relatively few regular osteons, the zonal BRUs are seen to make up more than one third of the total remodeled capsular area (4.47 m^2, versus 7.51 m^2 from normal BRUs), thus partially compensating for their smallness by their number.

The mean label area of the abortive BRUs (and the mean diameter, as well; not shown) increases until a distance of about 3,200 µm from the PLS. This relative diminution of the inhibition is not easily explained anatomically, since the most prominent histologic organ-level feature to explain such a functional partial limit would be the usually abrupt change from the endochondral layer to the outer periosteal layer. Since the functional data are summed up from many slices and since the distances to the PLS of this transition change all along the periphery of a given PLS in every slice, the registered change will not be as abrupt as the anatomic one. Nevertheless, histologically, this border is much closer to the PLS, often being between 400 and 1,200 µm away in the dog and thus not allowing an explanation of the apparent 3,200-µm centripetal inhibition. The alternative cause-effect relationship in the form of a functional inhibition emanating from the PLS, explaining the postponed removal of the fetal endochondral layer, is more plausible. However, it should not be forgotten that remodeling features are not only locally controlled, but submitted to systemic control, as indicated by the measurable interindividual variation in BTO involving the otic capsule, as well as the humerus (though not fully parallel; Fig 4). Most probably, the particular otic remodeling conditions should be conceived as a strong local modification of a general remodeling principle. Whether this measurable inhibition is a "field effect" originating from the PLS or is an innate peculiarity of the calcified tissue of the otic capsule has not been clarified. Considering the permanent electrical activity and huge potential differences maintained within the membranous inner ear, the existence of a powerful electromagnetic field close to the PLS and waning in inhibitory influence with distance is convincing as a model, but canalicular transduction of a signal through the interconnected osteocytic processes of capsular bone cells would also be consistent with these findings.

Whatever the governing principle, it is clear from the influence on the BRU level that it is affecting both the activation of the osteoclasts (ie, reduction in numerical density of all BRUs) and the resorptive activity of these cells (ie, reduction in size of zonal BRUs) with increasing proximity to the PLS. Thus, at least the 2 first parts of the activation-resorption-formation coupling in remodeling are affected, possibly explained by some influence on the cellular level. Local momentary loss of — or overcoming of — this inhibition may play a role as a possible pathway for onset of abnormal but sharply delimited remodeling, such as abortive BRUs and, in particular, otosclerosis. Our results indicate it may be worthwhile concentrating on the role of the cochlear and vestibular compartments in this inhibition.

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ISOLATED LARGE VESTIBULAR AQUEDUCT SYNDROME IN A FAMILY

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PALO ALTO, CALIFORNIA

This paper presents the second case in the literature of large vestibular aqueduct syndrome without associated cochlear anomalies in 2 members of the same family. The syndrome is frequently associated with sensorineural hearing loss presenting in childhood. The onset is commonly sudden, following an event causing increased intracranial pressure. On the basis of an emerging pattern of inheritance, we recommend screening siblings of an affected child. We also discuss the importance of characterizing the extent of disease of the inner ear.

KEY WORDS — familial hearing loss, hearing loss, large vestibular aqueduct syndrome, sensorineural hearing loss, sudden hearing loss.

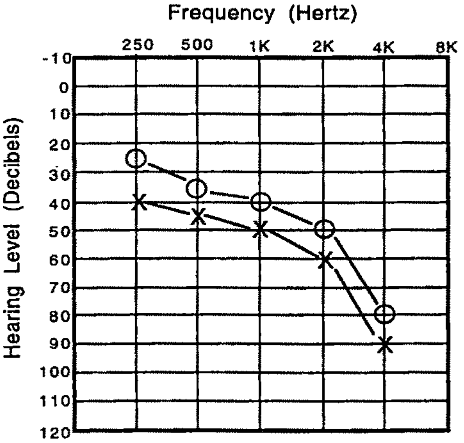
INTRODUCTION

Enlargement of the vestibular aqueduct as seen in radiographic studies, such as computed tomography (CT), is associated with sensorineural hearing loss, usually in early childhood. It is the second most common malformation of the inner ear.[1] The hearing loss is frequently progressive or fluctuating, and patients often experience sudden decreases in their hearing following minor head trauma or activities involving a Valsalva maneuver. Treatment of the hearing loss is symptomatic, and avoidance of inciting events is the only form of prevention.

Several case series have delineated the clinical characteristics of large vestibular aqueduct syndrome (LVAS).[1-8] Only 1 prior report exists, however, of familial isolated LVAS.[9] We present 2 siblings with LVAS and no cochlear disease, and review the associated literature. We emphasize the importance of considering isolated LVAS as a separate entity from LVAS with associated cochlear disorders. Additionally, we present LVAS as a familial condition to encourage screening of family members of an affected individual.

CASE REPORT

A 2½-year-old boy was referred for evaluation of possible hearing loss. The child's mother became concerned about a lack of speech development when the child reached the age of 2. Behavioral audiometry revealed a bilateral sensorineural hearing loss, moderate on the right and moderately severe on the left (Fig 1). The findings on tympanometry and otologic examination were normal. The auditory brain stem response (ABR) showed normal wave I-V interpeak latencies and a normal interaural wave V latency, but abnormally high thresholds at 70 to 80 dB. Subsequent audiometry confirmed the thresholds.



Key to Symbols

Air Conduction	Right	Left	Bone Conduction	Right	Left	Sound Field	S
Unmasked	O	X	Unmasked	<	>	Aided	A
Masked	Δ	□	Masked	[	]	No Response	↓

Summary

R	PTA	SDT	ST	WRS
	42	50		

L	PTA	SDT	ST	WRS
	52	35		

Fig 1. Initial patient's audiogram revealing bilateral hearing loss. PTA — pure tone average (in decibels); SDT — speech detection threshold (in decibels); ST — spondee threshold (in decibels); WRS — word recognition score (in percent).

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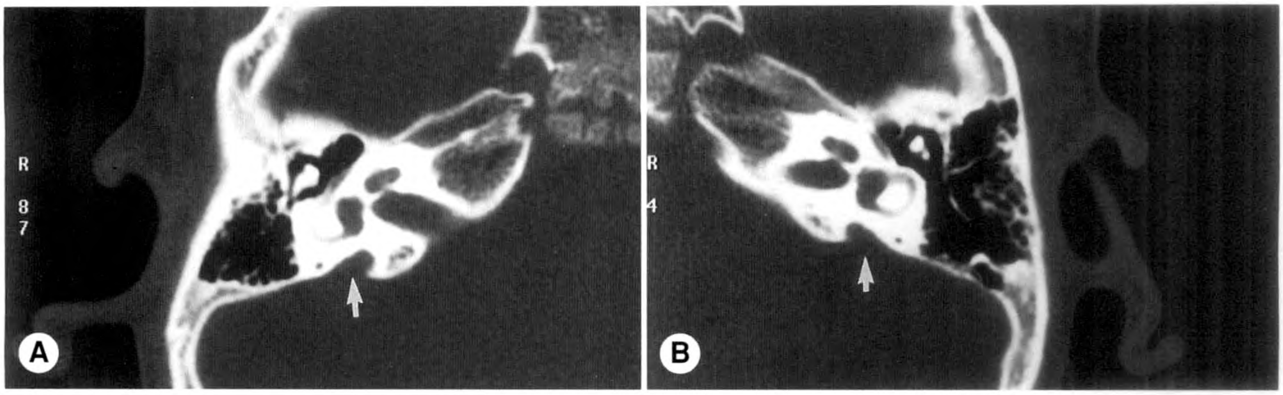


Fig 2. High-resolution computed tomography of initial patient, showing bilateral enlarged vestibular aqueducts (arrows) and vestibules (as defined by Arcand et al[5]) with normal cochlea and middle ear structures. A) Right side. B) Left side.

The child was fitted with bilateral hearing devices and referred for speech therapy.

The child was a product of normal gestation during which the mother received routine prenatal care and was not exposed to any known potential teratogens. The child had an uncomplicated delivery and was discharged from the hospital on the second day of life. He had no history of kernicterus, anoxia, ototoxic medication exposure, head trauma, or meningitis. At the time of his evaluation, his health had been unremarkable except for intermittent eczema, an umbilical hernia, and recurrent acute otitis media. Developmentally, he had met appropriate motor milestones, including independent ambulation at the age of 10½ months. His family history was notable for a maternal uncle and maternal grandmother incurring unilateral hearing loss early in childhood. The parents and 3 siblings denied any hearing loss at the time of presentation. His physical examination yielded unremarkable findings except for a scar related to his umbilical hernia repair.

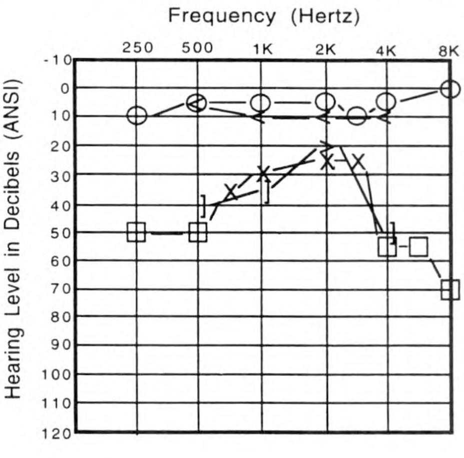
Given the strong family history of childhood hearing loss and the observed lack of cause for this child's hearing dysfunction, a bilateral temporal bone CT scan was obtained. The findings were consistent with LVAS (Fig 2[5]). The right and left vestibular aqueducts measured 3.1 and 3.8 mm, respectively, according to the technique of Valvassori and Clemis.[2] The patient's family was educated about LVAS, and instructions for the patient included avoidance of contact sports, heavy lifting, or straining. He was encouraged to have audiograms every 3 months, utilize his hearing aids, and continue speech therapy. No surgical intervention was offered.

Two months after the child's presentation, his 5-year-old sister underwent a hearing evaluation to rule out hearing loss. The family had never noted signs of a hearing loss, and her speech and language development had been normal. Pure tone audiometry revealed normal thresholds on the right and a moderate sensorineural loss on the left (Fig 3). This loss was consistent with subsequent audiograms and distortion product otoacoustic emissions.

Like her brother, she had no remarkable birth events or in utero exposures. She was healthy except for multiple urinary tract infections requiring cystoscopy and dilation. Findings on her physical examination were unremarkable, revealing no external or middle ear disease.

A temporal bone CT scan was obtained, demonstrating a large vestibular aqueduct (3.8 mm) and ves-

Fig 3. Audiogram of initial patient's sister, revealing normal hearing on right and sensorineural hearing loss on left.



Key to Symbols

Air Conduction	Right	Left	Bone Conduction	Right	Left	Sound Field	S
Unmasked	○	X	Unmasked	<	>	Aided	A
Masked	Δ	□	Masked	[	]	No Response	↓

Summary

R	PTA	SDT	ST	WRS
	5		10	100

L	PTA	SDT	ST	WRS
	10		30	84

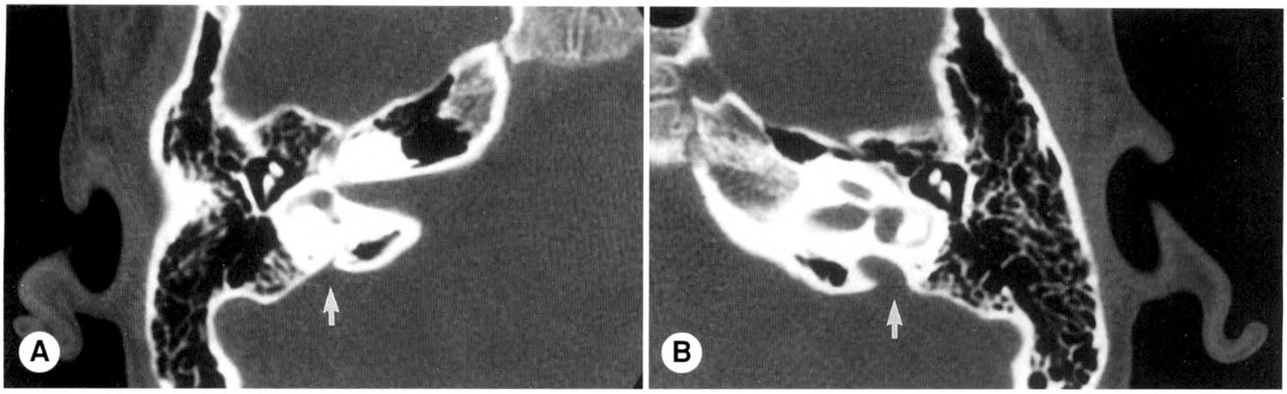


Fig 4. High-resolution computed tomography of initial patient's sister. A) Right side, with normal vestibular aqueduct (arrow) and other inner and middle ear structures. B) Left side, with enlarged vestibular aqueduct (arrow) and vestibule with normal cochlear and middle ear anatomy.

tibule on the left side (Fig 4). No other external, middle, or inner ear disease was identified.

DISCUSSION

Large vestibular aqueduct syndrome was first described clinically in 1978 by Valvassori and Clemis.[2]

[redacted]

ing loss. The clinical presentation has been described as a predominantly high-frequency hearing loss, beginning from birth to adolescence, with an acute onset and a fluctuating or progressive course. A review of the 8 major case series[1-8] shows onset primarily during childhood. There is a female predominance of up to 2:1.[6] Unilateral occurrence is less common physiology of LVAS. Jackler and De La Cruz[6] propose arrest of development of the vestibular aqueduct near the fifth week of gestation. This theory explains the large appearance of the aqueduct in terms of gestational development. Sudden pressure changes leading to membrane distention or rupture with resultant mixture of endolymph and perilymph have been postulated.[6,14] The large bony aqueduct may not be able to serve as a resistor to acute pressure fluctuations. Gradual distention of Reissner's membrane, producing hydrops, is less likely, given that hydrops preferentially affects the apex of the cochlea and has a low-frequency loss. Alternatively, hyperosmotic fluid of the endolymphatic sac may reflux into the cochlea via the widely patent vestibular aqueduct, resulting in damage to hair cells.[7] The large bony aqueduct may be a radiographic finding that is coincidental to a pathological membranous inner ear malformation.[4]

With the initial case series by Valvassori and Clemis[2] in 1978, LVAS was not considered to be a familial syndrome. However, Griffith et al[9] presented 2 male siblings with LVAS and normal parents. The proband had additional radiographic findings of large vestibules and small lateral semicircular canals. They concluded that the inheritance was probably autosomal recessive or X-linked. Two additional studies in the radiographic literature report familial cases of LVAS.[15,16] However, Mondini malformations were present in their patient populations, and the authors did not clarify the status of the other inner ear structures. These 2 latter studies suggest a genetic link in transmission of LVAS, although not with a malformation of the vestibular aqueduct alone.

Upon diagnosis of LVAS, the patient and family should be educated. Preventing hearing loss or the progression of an existing hearing loss is difficult. Most important is the avoidance of head trauma, the slightest of which may incite a sudden hearing loss. Counseling should include avoidance of contact sports; activities with large shifts in barometric pressure, such as scuba diving; and activities with a Valsalva, such as lifting. With the identification of a patient, family members should be screened with routine audiometry. Siblings to young adulthood, even without hearing loss, may be considered for screening with temporal bone CT.

Treatment of LVAS is primarily symptomatic. With hearing loss onset, hearing devices are prescribed as needed. Speech therapy, preferential seating, frequency modulation systems, and special schooling can be utilized as appropriate. Surgical intervention is controversial. Jackler and De La Cruz[6] performed an endolymphatic-subarachnoid shunt in 7 ears with LVAS and had an increased hearing loss in 4 ears postoperatively. As a result, they did not recommend shunting as a surgical intervention. Similarly, improvement was found by Reussner et al[17] in a patient on whom an endolympatic-mastoid shunt was performed. Wilson et al[14] performed endolymphatic sac obliteration on 7 ears in 6 patients with progressive hearing loss and LVAS. Two of the ears had improved hearing, 4 remained stable, and 1 had a progressive hearing loss. On the basis of these findings, the authors concluded that patients with LVAS and progressive hearing loss should be considered for such intervention, although this was disputed in a commentary following the article. A recent case report discusses 2 patients with successful cochlear implantation, although long-term follow-up is not presented.[18] Intraoperatively, small gushers were noted during the cochleostomies.

CONCLUSION

The presence of a radiographically large vestibular aqueduct is strongly associated with the sudden onset of sensorineural hearing loss in early childhood. Unfortunately, the diagnosis is usually made following the onset of the patient's hearing loss. This paper is the second in the literature to report a familial incidence of LVAS without associated cochlear disease. Given the probable genetic basis for this syndrome, family members should be screened with routine audiometry followed by radiographic studies as appropriate. As in our case report, it is important to identify whether an enlarged vestibular aqueduct is without associated cochlear abnormality, as this represents a separate clinical entity. An isolated enlarged vestibular aqueduct is associated with a sudden onset of hearing loss that is more severe in the high frequencies. This information is important for prognosis and preventative counseling to avoid head trauma or activities that increase intracranial pressure.

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REVERSIBLE SENSORINEURAL HEARING LOSS FOLLOWING ADMINISTRATION OF MUROMONAB-CD3 (OKT3) FOR CADAVERIC RENAL TRANSPLANT IMMUNOSUPPRESSION

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This prospective study is a follow-up to a case report noting reversible sensorineural hearing loss after administration of OKT3 for immunosuppression in a steroid-resistant renal cadaveric transplant patient who was rejecting his transplant. The objective is to determine the interval estimate for incidence of sensorineural hearing loss following treatment with OKT3. Seven patients were admitted to the Renal Transplant Service at Montefiore Medical Center from July 1996 to July 1997 with steroid-resistant rejection of renal cadaveric transplants and received OKT3 as an immunosuppressant. All 7 patients received 3 audiograms: the first, prior to the administration of the first dose of OKT3, the second, 48 to 72 hours after administration of OKT3, and the third, approximately 2 weeks after administration of OKT3. Five of the 7 patients (71%) demonstrated a sensorineural hearing loss of 15 dB or greater at frequencies of 8 to 12 kHz. Four of the 5 patients with audiographic changes had near-complete to complete recovery of their high-frequency thresholds after discontinuation of the drug regimen. In conclusion, OKT3 can cause sensorineural hearing loss. This side effect is mainly reversible after 2 weeks following discontinuation of the drug. Patients receiving OKT3 should be forewarned of this possible side effect prior to the administration of OKT3.

KEY WORDS — muromonab-CD3, OKT3, ototoxicity, sensorineural hearing loss.

INTRODUCTION

Reversible ototoxicity has been documented following the administration of various therapeutic agents, including salicylates[1] and erythromycin.[2] After noting a case of reversible sensorineural hearing loss following OKT3 (murine monoclonal antibody CD3; muromonab-CD3) administration,[3] we conducted a prospective study to determine the incidence of OKT3-induced hearing loss and its reversibility upon discontinuation of the therapeutic agent. OKT3 is a monoclonal antibody used as an immunosuppressant following cadaveric renal transplants that has been noted to have a host of adverse side effects but has not been previously documented to produce sensorineural hearing loss.

MATERIALS AND METHODS

From July 1996 to July 1997, 7 patients were admitted to the Renal Transplant Service for steroid-resistant rejection of cadaveric renal transplants. Each of the patients signed an informed consent according to the Montefiore Medical Center Institutional Review Board–approved protocol. All patients underwent questioning regarding baseline hearing loss, vertigo, or tinnitus and then underwent audiometric testing (in which normal ANSI standards were observed) from 250 Hz to 12 kHz. Approximately 72 hours after administration of the first dose of OKT3, a second audiogram was made and the patients were questioned again as to symptoms of hearing loss, tinnitus, or vertigo. The total course of treatment with OKT3 ranged from 5 to 8 days at a daily intravenous dosage of 5 mg. At least 2 weeks after the final dose of OKT3, a final audiogram was made. The exact timing of this third audiogram was somewhat variable, as several of the 7 patients became acutely ill during this time period and audiological testing was delayed by several days.

RESULTS

Five of the 7 patients in the present study demonstrated a sensorineural hearing loss in the range of 3 to 12 kHz 48 to 72 hours after administration of OKT3 (see Table). The mean sensorineural hearing loss for the 7 patients tested was 18 dB, with a standard deviation of 15 dB. When the 2 patients with prior hear-

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AUDIOLOGICAL FINDINGS

Patient	*Ear*	*Pretreatment*	*48 to 72 Hours After First OKT3 Treatment*	*2 Weeks After Cessation of OKT3 Treatment*
1	L	50 dB at 8 kHz	30 dB at 12 kHz, 100%	Returned to baseline
	R	Normal	40 dB at 12 kHz, 100%	Returned to baseline
2	L	70 dB at 12 kHz, 92%	30 dB at 8 kHz, 92%	25 dB at 8 kHz, 96%
	R	70 dB at 12 kHz, 96%	25 dB at 8 kHz, 96%	15 dB at 8 kHz, 96%
3	L	Normal	45 dB at 12 kHz, 100%	No change
	R	Normal	25 dB at 12 kHz, 96%	15 dB at 12 kHz, 92%
4	L	70 dB at 8-12 kHz, 100%	No change	Not performed
	R	70 dB at 3-12 kHz, 84%	No change	Not performed
5	L	Normal	15 dB at 4 kHz, 100%	Patient died
	R	Normal	No change	
6	L	45 dB at 8 kHz, 85 at 12 kHz, 92%	No change	No change
	R	30 dB at 8 kHz, 75 dB at 12 kHz, 96%	15 dB at 3 kHz, 88%	Returned to baseline
7	L	Profound sensorineural loss	No change	Not performed
	R	40 dB at 4 kHz, 60 dB at 12 kHz, 92%	No change	Not performed

Percentages are speech discrimination scores.

ing loss were excluded from the sample population, the calculated mean hearing loss was 25 dB, with a standard deviation of 10 dB. Two weeks following completion of the final dose of OKT3, 4 of the 5 patients who demonstrated hearing loss after treatment underwent a third audiogram. The fifth patient died during the 2 weeks after completion of the OKT3 regimen. All 4 of the patients tested showed near to complete recovery of their auditory threshold shifts (within 15 dB).

STATISTICS

Five of the 7 patients (71%) demonstrated sensorineural hearing loss following treatment. The estimated standard deviation for this population is calculated to be 0.17. In order to calculate the interval estimate in which we are 90% sure that the sample proportion of those affected by OKT3 resembles the population proportion, we used a chart of standard normal-tail probabilities to calculate a 90% confidence interval of 0.43 to 0.99. With the same table, the interval estimate that produces a 95% confidence interval is 0.38 to 1.00.

DISCUSSION

Ototoxicity from different drug regimens has been a well-known phenomenon since the 1800s, when treatment with quinine or acetylacetic acid was noted to produce symptoms of dizziness, tinnitus, and hearing loss.[4] Modern analysis of ototoxicity was spurred in the 1940s by the widespread use of streptomycin in treating tuberculosis. The family of known ototoxins has slowly expanded over the years and currently includes members of the aminoglycoside family, loop diuretics, cis-platinum, and nitrogen mustard in addition to quinine and salicylates. Of these ototoxins, some are preferentially vestibulotoxic, while others are cochleotoxic. Ototoxicity itself has been most commonly measured as a decrease in threshold of 10 dB in both ears or at least 15 dB in 1 ear.[5] Depending on the mechanism of action of the specific agent, the hearing loss or vestibular disorder may be temporary or permanent.

The auditory effect of OKT3 was first noted after a patient became symptomatic with nonpulsatile tinnitus and hearing loss several days after receiving OKT3 treatment.[3] The patient had no baseline audiogram for comparison, but the symptoms themselves were dramatic. A subsequent audiogram performed approximately 2 weeks after the drug regimen was finished showed a near-complete reversal of the hearing loss. Upon questioning, the patient revealed that the hearing loss and tinnitus had dramatically improved. Based upon this anecdotal finding, a prospective study was designed. The first, baseline audiogram was made just after the renal biopsy and just prior to the first administration of OKT3, whereafter the only variable was the administration of OKT3. The second audiogram was obtained at the time point at which the first patient first noted a subjective loss. The third audiogram, made 2 weeks after the completion of the drug regimen, was designed to reflect a time point in the hearing recovery. However, as all 4 of the living patients who suffered temporary hearing loss following treatment had near-complete resolution by this time point, it may represent the time required for recovery.

It is worthy of note that the 2 patients who did not experience hearing loss following treatment with OKT3 had documented baseline sensorineural hearing loss. The overall interval estimate of incidence was calculated with these 2 patients included in the sample population. The data with reference to mean sensorineural hearing loss and standard deviation were then calculated by first including and then excluding these 2 patients to suggest what the mean and standard deviation would be if they were considered as variants. It may well be that people with preexisting hearing loss may not be vulnerable to temporary hearing loss induced by OKT3. This may be borne out by future research.

OKT3 is a murine monoclonal antibody used in the acute rejection of renal allografts. This antibody reacts with the CD3 portion of a surface molecule on thymocytes and mature human T-cells.[6] Once bound to OKT3, the CD3+ cells are opsonized, and subsequently removed by the reticuloendothelial system.[7] OKT3 has been found to significantly reduce the rate of renal rejection[8]; however, its usage has been hampered by its association with serious side effects ranging from rashes to nausea and vomiting to high fevers, seizures, and anaphylaxis. Nevertheless, until better drug regimens can be developed that offer similar efficacy against renal transplant rejection, OKT3 will continue to be used.

In our series, 5 of the 7 patients who received OKT3 suffered high-frequency sensorineural hearing loss following treatment, mostly in the range from 8 to 12 kHz. This is consistent with the type of hearing loss produced by other ototoxins such as kanamycin or amikacin, in which selective destruction of the outer hair cells at the basal turn of the cochlea occurs as the initial event and produces a similar pattern of hearing loss.[4] Of the 5 patients who experienced hearing loss, 2 noted experiencing tinnitus. The findings of 2 patients whose audiological test results showed no change after treatment are difficult to interpret, as they had baseline high-frequency sensorineural hearing loss that may have masked the effect.

The mechanism of how OKT3 produces ototoxicity remains unclear. Cellular models for ototoxins producing a similar pattern of hearing loss have been described. Aminoglycosides, for example, first produce a reversible effect by which ion transport is blocked, and later result in permanent cell death by binding to phospholipids and impairing protein synthesis.[9] Loop diuretics have been described as producing a deactivation of sodium-potassium ATPase, thus producing a change in the endocochlear potential.[10] Cis-platinum produces a degeneration of the outer hair cells at the basal turn of the cochlea.[4] Quinine produces variable damage to the organ of Corti and produces strial atrophy.[4] Salicylates affect enzyme function within the hair cells and cochlear neurons.[4] As new ototoxins such as OKT3 emerge, further characterization of their cellular mechanism of action may shed light on the problem of ototoxicity and may allow for further understanding with regard to possible prevention and treatment.

In summary, OKT3 is an important drug in the arsenal aimed at preventing renal transplant rejection; its dosage must be titrated toward its central aim. Knowledge of the potential side effects (and, importantly, of their duration) may not help in avoiding the untoward effects of a given drug, but it may serve to better inform patients receiving the treatment of what they may experience.

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SCHWANNOMA OF THE CERVICAL SYMPATHETIC CHAIN

THE VIRGINIA EXPERIENCE

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We present 4 cases of schwannomas arising from the cervical sympathetic chain. These lesions are uncommon and most often present as an asymptomatic solitary neck mass. Preoperative diagnosis can be difficult, even with the aid of computed tomography, magnetic resonance imaging, ultrasound, and angiography. While a paraganglioma can often be ruled out, exact determination of the nerve of origin is frequently elusive until the time of surgery. Operative excision remains the treatment of choice, often requiring sacrifice of a portion of the sympathetic chain. Postoperative Horner's syndrome is common, but does not appear to have an adverse effect on the patient.

KEY WORDS — neurilemoma, schwannoma, sympathetic nerve.

INTRODUCTION

Schwannomas arising from the cervical sympathetic chain are uncommon, as reflected by the presence of only sporadic case reports in the literature.[1-3] Like most schwannomas, they are most often solitary, benign, and slow-growing,[4,5] with rare malignant degeneration.[6] Patients commonly present with an otherwise asymptomatic neck mass, with Horner's syndrome often not present on physical examination.[7]

At the University of Virginia between 1993 and 1997, 4 patients were treated for schwannoma of the cervical sympathetic chain. We present these cases with particular emphasis on symptomatology and available diagnostic imaging studies that may assist in making an accurate diagnosis. As the initial symptom complex and treatment course were similar for each of our patients, we will discuss in detail 1 case as representative of the group.

CASE REPORT

A 60-year-old man presented with an asymptomatic right neck mass found on routine physical examination. Measuring approximately 4 × 5 cm, this mass lay medial to the sternocleidomastoid muscle, at the level of the carotid bifurcation. It was mobile, nontender, and nonpulsatile, with no associated bruit. The right pupil appeared slightly smaller than the left, but no ptosis was noted. The vocal fold mobility remained intact. The remainder of the head and neck examination yielded normal findings. Fine needle aspiration revealed spindle-shaped cells with irregular fusiform nuclei in a vague interwoven pattern. However, a definitive diagnosis could not be made. Diagnostic studies included computed tomography (CT) and magnetic resonance imaging (MRI) confirming a well-circumscribed mass in the poststyloid parapharyngeal space on the right that displaced the carotid artery anteriorly and the internal jugular vein laterally (Fig 1). The mass was slightly heterogeneous, with high signal intensity on T2-weighted images and moderate enhancement postcontrast. In addition to sympathetic schwannoma, the preoperative differential diagnosis included vagal schwannoma, metastatic or reactive lymphadenopathy, and paraganglioma.

The patient was taken to the operating room, where the mass was exposed through a transverse cervical incision. The tumor was found to clearly arise from the sympathetic chain. The mass was unable to be resected without sacrificing a portion of the chain. Schwannoma was confirmed by pathology. Postoperatively, the patient exhibited mild right miosis without ptosis, which is resolving slowly.

DISCUSSION

A schwannoma of the cervical sympathetic chain most often presents as an asymptomatic solitary neck mass in the poststyloid parapharyngeal space on imaging studies and is associated with a minimum of physical findings.[7] In a review of the literature, Sheridan and Yim[8] report an age range between 5 and 66 years, with a 3:1 male-to-female ratio. They found other possible presenting symptoms to include dysphagia, change in voice, hearing loss, and cranial

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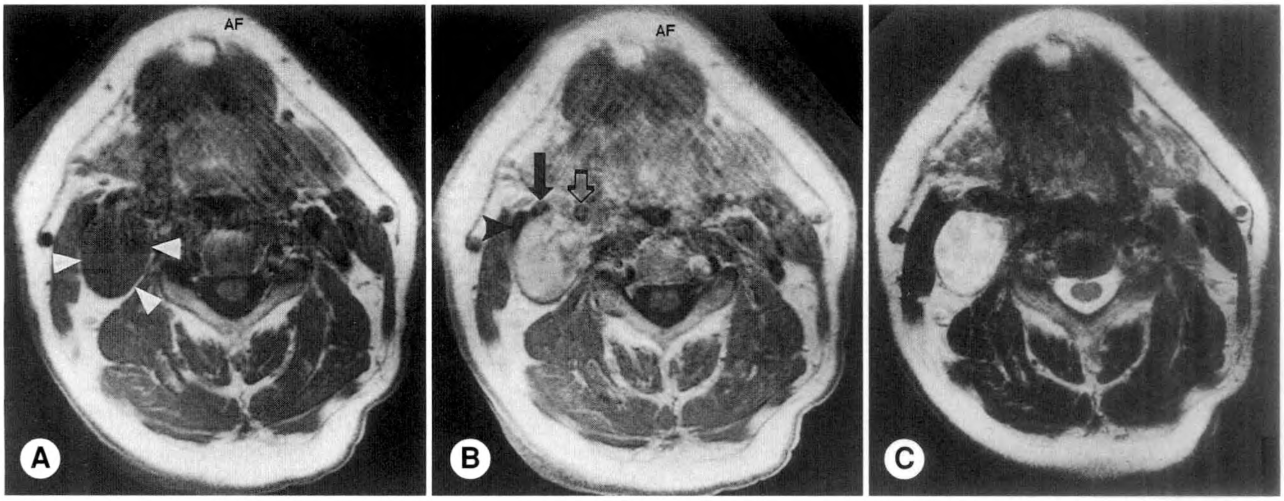

Fig 1. Axial magnetic resonance images of right cervical sympathetic chain schwannoma. A) Precontrast T1-weighted image shows well-circumscribed 4 × 5-cm, slightly heterogeneous mass (arrowheads) deep to sternocleidomastoid muscle. B) Postcontrast T1-weighted image demonstrates moderate enhancement of mass, with anterior displacement of internal carotid artery (open arrow) and external carotid artery (closed arrow) and lateral compression of internal jugular vein (arrowhead). C) T2-weighted image reveals high signal intensity of mass.

nerve palsies, and found preoperative Horner's syndrome to be rare. In our series, ages ranged from 20 to 60 years. None of the 4 patients presented with a complete Horner's syndrome, and only 1 patient demonstrated ipsilateral miosis. No patient complained of compressive symptoms associated with the mass.

The differential diagnosis of a unilateral parapharyngeal space mass is based on the division of this space into prestyloid and poststyloid compartments, with possible tumor causations unique to each space. Masses arising in the prestyloid compartment may include deep lobe parotid tumors, lipomas, lymphadenopathy, and rare neurogenic tumors. In the poststyloid compartment, possible causes include carotid artery aneurysms, neurogenic tumors involving cranial nerves IX through XII, as well as the sympathetic chain, and paragangliomas arising from the vagus nerve or carotid body. Several uncommon tumors, such as meningioma,[9] lymphoma,[10] and teratoma,[11] have been described in the parapharyngeal space. Fine needle aspiration, while conclusive in many cases of neck masses, is much less valuable for the compact neural tumor, and failed to reveal the diagnosis in both of our patients in whom it was performed.

Imaging studies play a central role in the diagnosis of the head and neck schwannoma. The most important task is to distinguish between a vagal or sympathetic schwannoma and a paraganglioma. Regardless of the nerve of origin, schwannomas in general are hypodense with comparison to muscle on CT without contrast.[12] With contrast, these lesions show at least some degree of enhancement, often at the periphery.[12] Paragangliomas, on the other hand, are classically isodense when compared to muscle on precontrast CT, with more reliable homogeneous enhancement postcontrast.[12] Magnetic resonance imaging of schwannomas reveals relatively low signal intensity on T1-weighted images and high signal intensity on T2-weighted images.[13] These tumors tend to enhance homogeneously with contrast.[13] Paragangliomas show extremely bright contrast enhancement on postgadolinium MRI sequences in a characteristic "salt-and-pepper" pattern, representing the low signal intensity of vascular flow voids.[13] However, this finding is not pathognomonic for paraganglioma and may be found with other hypervascular lesions.[13]

With significant enhancement, a vascular lesion must be ruled out. The angiographic appearance of schwannoma has been characterized by Abramowitz et al,[14] who describe a vascular pattern consisting of scattered "puddling" of contrast medium throughout the lesion, with no evidence of arteriovenous shunting. The relationship of the mass to the surrounding vascular structures is important in ruling out a carotid body tumor. Classically, a carotid body tumor will splay the internal and external carotid arteries, producing the "lyre sign" (Fig 2). This is rarely associated with a schwannoma.[3]

Perhaps the most difficult task is to determine the nerve of origin. Schwannomas may arise from any of the neural structures of the parapharyngeal space, including cranial nerves IX, X, XI, and XII.[15] Furukawa et al[16] found that schwannomas of the vagus nerve often cause a separation between the internal jugular vein and the internal or common carotid artery, while those arising from the cervical sympathetic chain do not. They also found that diagnosis of

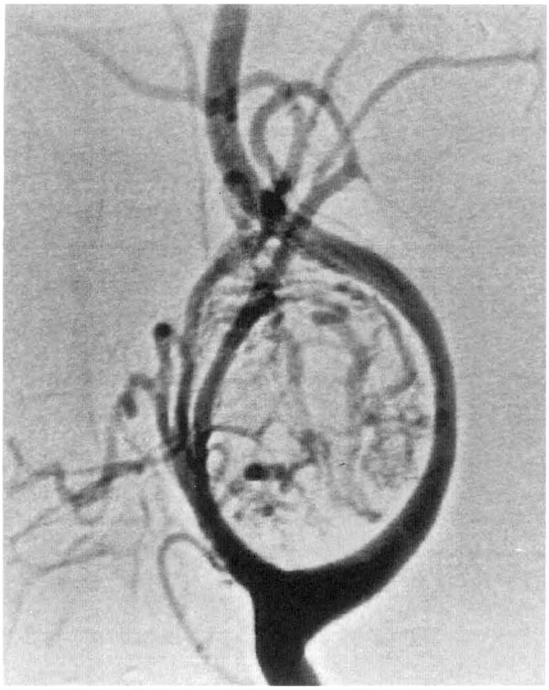

Fig 2. Lateral angiographic view of common carotid artery demonstrates classic "lyre sign" with splaying of internal and external carotid arteries by carotid body tumor.

the nerve of origin can often be made by ultrasound, since the vagus nerve can be identified, while the sympathetic chain cannot. The vagus nerve will either appear to course through the tumor (vagal schwannoma), or appear to pass around it (sympathetic chain schwannoma). In our study, all 4 patients underwent MRI, 2 underwent CT, and 1 each underwent ultrasound and angiography. On the basis of these tests, all 4 patients were thought to have schwannomas, but the nerve of origin was misdiagnosed as vagal in 3 patients, and in the remaining patient it could not be clearly ascertained until surgery.

Abramowitz et al[14] report preoperative embolization of head and neck schwannomas to be a relatively safe and effective adjunct to surgical resection in cases of more vascular tumors. Each of the patients in our series underwent surgical resection without preoperative embolization. In each, the vagus nerve was clearly identified and protected, revealing the origin of the tumor. Classically, a neurofibroma will be intimately associated with the nerve of origin, while a schwannoma will arise eccentrically and thus be more amenable to surgical sparing of the nerve.[17] However, this was not our experience in this series of patients. The mass was not able to be dissected from the sympathetic chain, due to intimate involvement with the nerve, requiring resection of a segment of the sympathetic chain. Postoperatively, all 4 patients developed either partial or complete Horner's syndrome. However, this caused no difficulties for the patients, and in each case showed partial resolution by the first postoperative visit, although 1 patient displayed persistent postoperative ipsilateral true vocal fold paralysis.

Pathologically, schwannomas will demonstrate 2 distinct areas of cellularity (Fig 3). Antoni A regions are composed of more densely arranged cells with specific areas of palisading nuclei arranged in rows known as Verocay bodies. Antoni B regions tend to be more hypocellular, with a more loose and disorderly arrangement. The proportion of these regions varies with each tumor.[18] Fortunately, malignant degeneration is rare.[6]

CONCLUSION

Schwannomas of the cervical sympathetic chain

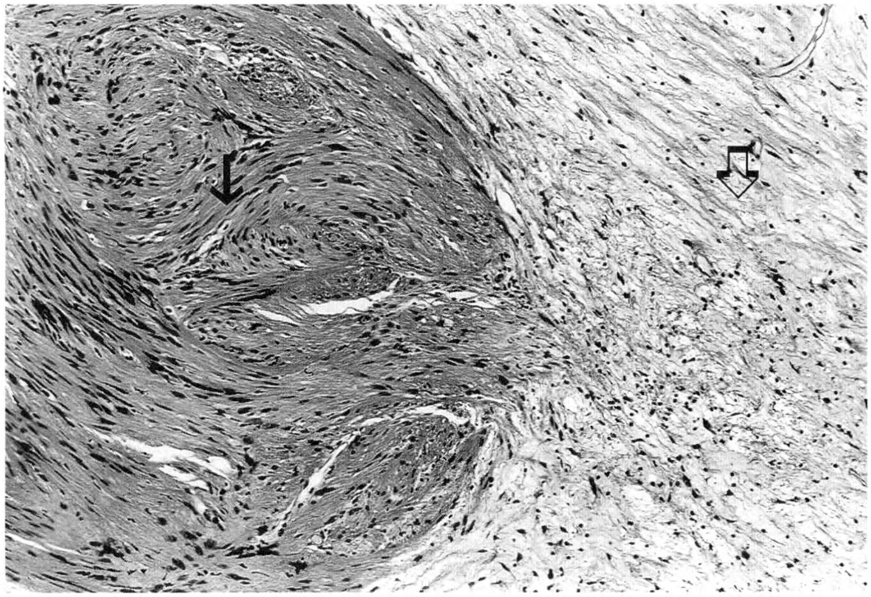

Fig 3. Hematoxylin and eosin–stained photomicrograph (original ×150) of sympathetic chain schwannoma. Antoni A region (closed arrow) shows dense arrangements of cells and palisading nuclei. Antoni B region (open arrow) is more loosely organized and hypocellular.

are rare neoplasms that most often present as asymptomatic unilateral neck masses. Definitive preoperative diagnosis may be difficult despite a variety of available imaging modalities, although it is often possible to rule out the presence of a paraganglioma such as a carotid body tumor. Such a finding would alter the treatment approach, with a probable need for preoperative embolization. Determination of the nerve of origin often remains elusive, even with the combination of CT, MRI, and ultrasonography. Diagnosis is often not made until the time of surgery. In this series of 4 patients, the tumor was intimately involved with the nerve, requiring partial resection of the sympathetic chain. Postoperative Horner's syndrome is common, but does not appear to cause problems, and usually at least partially resolves with time.

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NUCHAL FIBROMA: A CLINICOPATHOLOGICAL REVIEW

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Nuchal fibroma, or collagenosis nuchae, is a benign soft tissue tumor that arises from the posterior cervical subcutaneous tissue, with a predilection for the interscapular and paraspinal regions. Because of its benign clinical course and its close histopathologic similarity to other benign head and neck lesions, this lesion may be misdiagnosed and underreported. The purpose of this paper is to review the histopathologic and radiologic findings unique to nuchal fibroma, and compare and contrast it to the other soft tissue neoplasms within the clinical differential diagnosis. These include several benign (elastofibroma, lipoma, fibrolipoma, nodular fasciitis) and rare malignant entities (fibrosarcoma, liposarcoma, fibromatosis).

KEY WORDS — benign neck mass, collagenosis nuchae, nuchal fibroma, posterior cervical mass, soft tissue tumors.

CASE REPORT

A 40-year-old man with an 11-year history of a midline posterior neck mass presented with a 2-month history of right postauricular pain. Ten years prior to presentation, an incisional biopsy performed at an outside hospital was interpreted as showing a desmoid tumor. The patient denied recent changes in the size of the mass, history of abnormal scar formation, previous neck trauma, or constitutional complaints. His physical examination findings were otherwise unremarkable. A magnetic resonance imaging (MRI) scan performed preoperatively showed a nonenhancing subcutaneous soft tissue mass of low intensity on both T1- and T2-weighted images (Fig 1). Excisional biopsy revealed a firm, white, ellipsoid, rubbery mass measuring 4 × 3 × 2 cm, with a clear tissue plane between the lesion and the underlying muscle. However, infiltration of the overlying dermis was evident and required excision of an island of normal skin. The wound was closed primarily without tension. Histopathologic examination revealed an unencapsulated, dense fibrous tissue mass composed of hypocellular collagen and focal elastic fibers consistent with the diagnosis of nuchal fibroma (Fig 2). At 2½ years' follow-up, the patient remains free of recurrence.

DISCUSSION

Since the first description of nuchal fibroma in the literature by Enzinger and Weiss[1] in 1988, a total of 15 patients with this entity have been reported in the English-language literature. However, this entity has not been addressed in the otolaryngological literature. Of these 15 patients, gender was specified in 13 patients, with a male preponderance noted in the majority of cases (10/13 or 77%). The ages ranged from 19 to 54 years (median 43 years, mean 39 years). Eleven nuchal fibromas (73%) were localized in the midline posterior cervical region. All were managed by surgical resection, with only 1 instance of recurrence.

Nuchal fibroma is a benign fibrous tumor that typically presents in the dorsal region of the upper neck. It has a predilection to occur in the interscapular and paraspinal regions around the cervical spine.[2] Nuchal fibroma often presents clinically as an indolent process characterized by a slow-growing, asymptomatic mass in the dorsal cervical region. When the mass is symptomatic, pain is the most common complaint (27%). It is rare for the patient to report changes in the consistency or character of the mass. Findings on physical examination include a nontender, mobile mass in the subcutaneous tissues of the posterior neck or occipital region.[2,3] The remainder of the findings on physical examination are generally unremarkable. Restriction of the cervical spine and upper limb range of motion has been reported in 1 case.[4]

Fine needle aspiration, although not previously described in the literature, may be useful in differentiating a benign lesion from a malignant one preopera-

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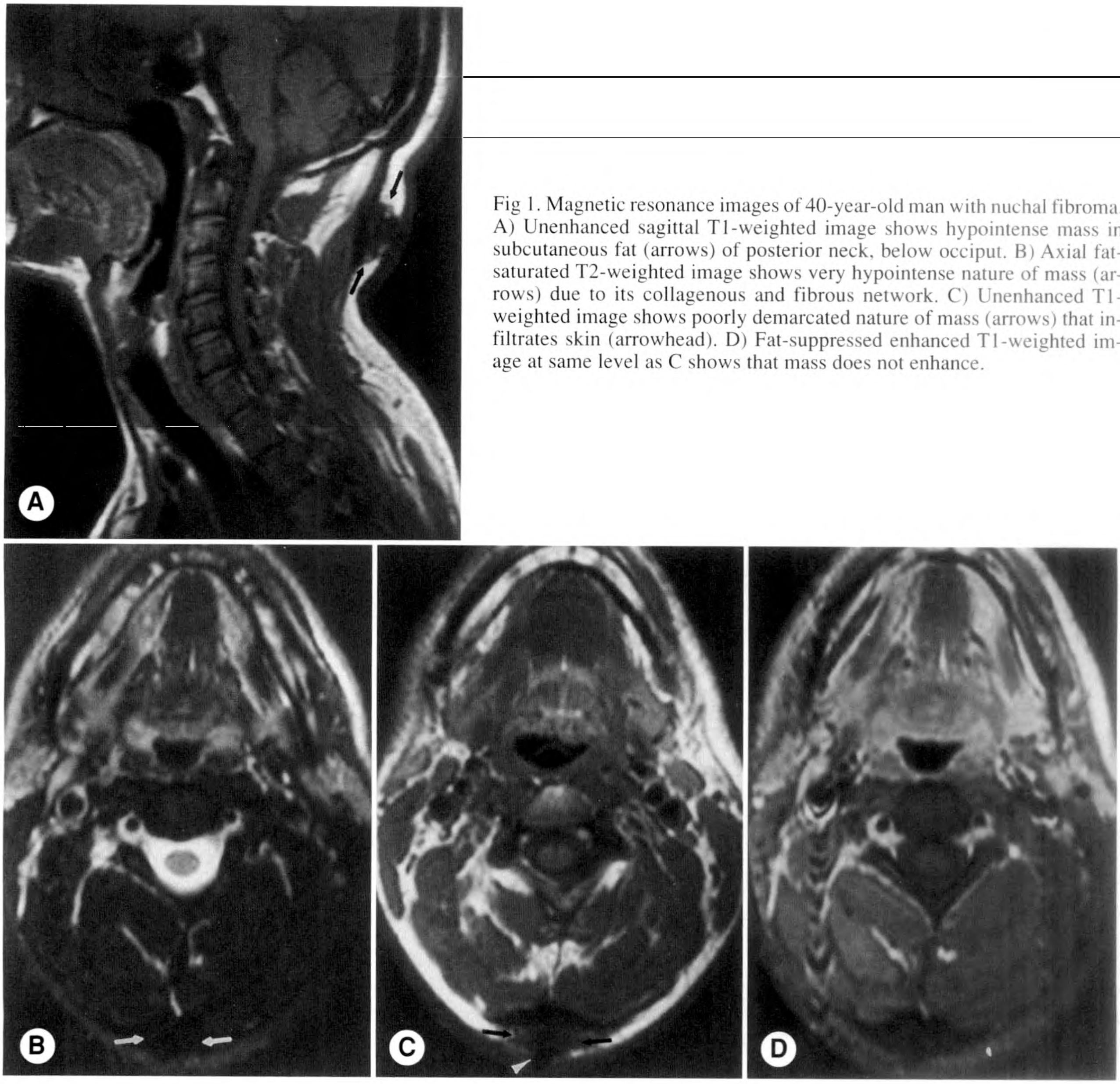

Fig 1. Magnetic resonance images of 40-year-old man with nuchal fibroma. A) Unenhanced sagittal T1-weighted image shows hypointense mass in subcutaneous fat (arrows) of posterior neck, below occiput. B) Axial fat-saturated T2-weighted image shows very hypointense nature of mass (arrows) due to its collagenous and fibrous network. C) Unenhanced T1-weighted image shows poorly demarcated nature of mass (arrows) that infiltrates skin (arrowhead). D) Fat-suppressed enhanced T1-weighted image at same level as C shows that mass does not enhance.

tively. However, the relative hypocellularity of this lesion may result in an "unsatisfactory" diagnosis due to lack of diagnostic material. This situation is analogous to that found in benign nerve sheath tumors.[5]

Radiographic evaluation is complementary to clin-

[redacted]

fibrous nature of these lesions is readily identified by MRI. However, while MRI may not be able to differentiate nuchal fibroma from other benign fibrous lesions, malignant fibrous lesions such as fibrosarcoma may sometimes be distinguished due to the presence of enhancing soft tissue within them.

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gressive manner. Patients typically present with a painless mass in the head and neck, without any known predilection for the dorsal cervical region. Fibrosarcomas have been associated with both prior radiotherapy and thermal burn injuries. Histologically, fibrosarcoma is composed of spindle-shaped cells with scant cytoplasm that grows in a fasciculated pattern. Given its aggressive clinical behavior and histopathologic pattern, fibrosarcoma can easily be differentiated from nuchal fibroma. Liposarcoma may also present as an enlarging, painless neck mass. Histologically, it consists of an encapsulated, lobulated mass scattered in mature fat cells (lipoblasts) characterized by pleomorphic nuclei; a rich capillary vascular network is also present, separating this lesion from nuchal fibroma.[10]

Fibromatosis, or extra-abdominal desmoid tumor, can also present in the neck. Grossly, this is an infiltrative mass freely interdigitating with skeletal muscle. Histologically, focally hypercellular areas composed of bland-appearing fibroblasts alternate with relatively acellular zones.[11] The infiltration pattern and muscle invasion separate this lesion from the benign nuchal fibroma.

Wide local surgical excision is the treatment of choice for nuchal fibroma. Of the 15 reported cases, only 8 (53%) had reported long-term follow-up (mean 70 months, range 6 months to 19 years). Of these 8 cases, 1 case of recurrence was reported with 19 months of follow-up. The authors attributed this "recurrence" to an incomplete excision of the mass. In our case, wide surgical excision has resulted in no evidence of recurrence at 2½ years of follow-up. However, the true incidence of recurrence of this slow-growing tumor will require additional long-term follow-up.

CONCLUSION

Nuchal fibroma is a benign soft tissue tumor that arises in the subcutaneous tissues of the posterior neck and occiput. Its indolent clinical course, as well as its close resemblance to other benign head and neck lesions, makes the clinical diagnosis of nuchal fibroma a difficult task. The histopathologic findings of nuchal fibromas are the hallmark of this lesion. We believe that this lesion may be underdiagnosed and underreported. Its true incidence and recurrence after surgical excision are unknown. Otolaryngologists should consider this lesion in the differential diagnosis of soft tissue masses arising in the dorsal cervical region. We recommend wide surgical excision of these lesions for diagnosis and management.

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DENDRITIC CELLS IN SELECTED HEAD AND NECK TUMORS

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Specimens from 17 head and neck tumor patients were immunohistochemically stained with monoclonal antibodies against HLA-DR, CD1a, RFD1, LAG, CD3, CD4, CD8, CD45RO, CD68, and cytokeratin to identify the nature and distribution of dendritic cells (DCs), T cells, and macrophages. Small numbers of DCs were present in all but 2 specimens. They were located between the tumor cells and in the stroma, especially in areas of inflammatory cell infiltration. Variable numbers of T lymphocytes (cytotoxic and memory type) occurred in the same locations. Numerous macrophages were found in the epithelium, in the stroma, and in the vicinity of tumor cells. The presence of DCs in head and neck tumors indicates that the organism has activated the immune surveillance system and is trying to present tumor antigens. Considering the sparsity of DCs in the malignant tissues, the T cell response can be only limited.

KEY WORDS — CD1a, dendritic cells, head and neck tumors, Langerhans cells, macrophages, RFD1, T cells.

INTRODUCTION

Dendritic cells (DCs) are potent antigen-presenting cells with the ability to initiate primary immune responses.[1] They populate lymphoid and many nonlymphoid organs such as the epidermis and other epithelia, including epithelia of the head and neck area.[2-4] In nonlymphoid organs, DCs are present in an immature state, whereas in the lymphoid organs, they occur both in an immature state and in a mature state. These states of differentiation are best studied with epidermal Langerhans cells (LCs) as a model. Immature DCs are "dendritic" in situ, and they have a very elaborate capacity to capture and process foreign protein antigens.[1,5] Inflammatory stimuli[6] and chemotactic factors[7] activate immature DCs. This activation leads to migration from nonlymphoid tissues via the afferent lymph[8] to the T cell areas of the lymphoid organs,[9] where DCs appear as mature. Mature DCs in culture or in lymph vessels are characterized by highly motile cytoplasmic processes ("veils"). They express high levels of surface major histocompatibility complex (MHC) class I and II molecules and adhesion and costimulatory molecules.[10,11] Mature DCs have a strong stimulatory capacity for resting T lymphocytes. They appear to be the only type of antigen-presenting cell that is able to activate naive, "virgin" T cells, ie, to initiate primary immune responses.[1] Mature DCs can also activate CD8$^+$ cytotoxic T cells directly, without the help of CD4$^+$ T cells.[12]

Tumor immunity is critically dependent on DCs.[13] The effector cells in tumor immunity are CD8$^+$ cytotoxic T cells[14] that recognize and kill tumor cells. They target the tumor cells' MHC class I molecules loaded with a peptide derived from tumor-specific antigens.[15] The failure to mount effective antitumor responses may have several causes. The DCs in tumors may be deficient in important T cell costimulatory molecules.[16] The density of tumor-infiltrating DCs may be too low.[4,17] The DCs may be inhibited in their function by immunosuppressive cytokines produced by the tumor cells, eg, interleukin-10.[18] It is the purpose of this study to extend these observations and to investigate in more detail the density and distribution of DCs in head and neck tumors.

MATERIALS AND METHODS

Specimens from 17 patients with malignant head and neck tumors, including 13 epidermoid carcinomas, 2 adenocarcinomas, 1 mucoepidermoid carcinoma, and 1 adenoid cystic carcinoma, were removed in the course of diagnostic biopsies or therapeutic tumor resections. They were histologically and immunohistochemically studied by using monoclonal antibodies (mAbs) and the avidin-biotin enzyme complex (ABC) method to identify DCs and LCs.

Fresh specimens of head and neck tumors were cut into small tissue blocks (5 × 5 × 5 mm) and snap-frozen in liquid isopentane in a freezer at −78°C. After transfer to a cryostat (Jung CM 3000, LEICA, Wetzlar, Germany) they were cut into 5- to 7-μm sections. These cryostat sections were collected on clean 3-aminopropyltriethyl-oxy-silane–coated glass slides

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TABLE 1. REACTIVITY OF HEAD AND NECK TUMORS WITH MONOCLONAL ANTIBODIES IDENTIFYING DENDRITIC CELLS, LANGERHANS CELLS, T CELLS, AND MACROPHAGES

Case No.	*Region*	*Histopathology*	*HLA-DR*	*CD1a*	*RFD1*	*LAG*	*CD3*	*CD4*	*CD8*	*CD45RO*	*CD68*
1	Paranasal sinus	Epidermoid carcinoma	–	+	–	+	+	+	+	+	+
2	Paranasal sinus	Adenocarcinoma	+	+	+	+	+	+	+	+	+
3	Paranasal sinus	Adenocarcinoma	+	+	+	ND	+	+	+	+	+
4	Parotid gland	Mucoepidermoid carcinoma	–	–	–	ND	–	–	–	–	+
5	Hard palate	Adenoid cystic carcinoma	+	+	+	ND	+	–	–	+	+
6	Base of tongue	Epidermoid carcinoma	+	+	+	ND	+	+	+	+	+
7	Base of tongue	Epidermoid carcinoma	+	+	+	–	+	+	+	+	+
8	Floor of mouth	Epidermoid carcinoma	+	+	+	ND	+	–	–	+	+
9	Pyriform sinus	Epidermoid carcinoma	+	+	+	ND	+	+	+	+	+
10	Pyriform sinus	Epidermoid carcinoma	+	+	+	+	+	–	+	+	+
11	Pyriform sinus	Epidermoid carcinoma	+	+	+	+	+	+	+	+	+
12	Hypopharynx	Epidermoid carcinoma	+	+	+	–	+	+	+	+	+
13	Subglottis and anterior commissure	Epidermoid carcinoma	+	–	–	–	+	+	–	+	+
14	Subglottis and anterior commissure	Epidermoid carcinoma	+	+	+	–	+	+	+	+	+
15	Supraglottis	Epidermoid carcinoma	+	+	–	ND	+	–	+	+	+
16	Supraglottis	Epidermoid carcinoma	+	+	–	ND	+	+	+	+	+
17	Supraglottis	Epidermoid carcinoma	–	+	–	+	+	+	+	+	+

+ — Presence of immunoreactive cells; – — absence of immunoreactive cells; ND — not done.

and air-dried for 24 hours. On the next day, cryostat sections were fixed in absolute ethanol at +4°C for 10 minutes, air-dried for 10 minutes, and incubated overnight at +4°C with primary mouse mAbs. The following antibodies were used: HLA-DR (diluted 1:100), OKT6/CD1a (undiluted hybridoma supernatant), RFD1[19] (1:100), LAG[20] (1:2), CD3 (1:100), CD4 (1:25), CD8 (1:25), CD45RO (1:100), CD68 (1:100), cytokeratin (1:100), and control IgG1, IgG2a, and IgM (each at the same concentration as the respective test antibody). The OKT6/CD1a-producing hybridoma cells were purchased from the American Type Culture Collection, Rockville, Md; LAG was a kind gift of Dr S. Imamura, Kyoto University, Kyoto, Japan[20]; mouse mAbs against HLA-DR, CD3, CD45RO, CD68, cytokeratin, IgG1, IgG2a, and IgM were from DAKO, Glostrup, Denmark; RFD1 was from Serotec, Oxford, England; and CD4 (Multi-Clone Anti-Leu-3a + Anti-Leu-3b) and CD8 (Anti-Human Leu-2a) were from Becton-Dickinson, Mountain View, Calif. For dilution, phosphate-buffered saline (PBS) containing 1% bovine serum albumin was used. After incubation with primary antibodies, cryostat sections were washed in PBS (3 × 10 minutes at +37°C). Thereafter, slides were incubated with biotinylated sheep anti-mouse Ig species-specific whole antibody (Amersham International, England), diluted 1:200, for 60 minutes at +37°C. Then, the cryostat sections were washed in PBS again (3 times) and incubated for 60 minutes at +37°C with streptavidin–horseradish peroxidase–conjugated complex (Amersham) at a dilution of 1:100. After washing in PBS (3 times), the peroxidase complex was visualized with 0.005% amino-ethyl carbazole in acetate buffer. After another washing step in PBS (2 times), the cryostat sections were washed with distilled water and counterstained with Harris' hematoxylin. Finally, the cryostat sections were rinsed with tap water and coverslipped with Kaiser's glycerin gelatin. As positive controls, cryostat sections from tonsil tissue were immunostained in parallel.

RESULTS

Immunohistochemical Examination With Cytokeratin. Specimens of all 17 cases of head and neck tumors were stained with the cytokeratin mAb. This antibody reacts with the great majority of benign and malignant epithelial lesions, including epidermoid carcinomas and adenocarcinomas. In all 17 cases, tumor cells reacted positively with the cytokeratin antibody. Negative controls on tonsil tissue showed no immunostaining with the IgG1 mAb. Positive controls showed strong immunostaining with cytokeratin in all simple and stratified squamous epithelia.

Distribution of DCs in Tumor Specimens. Specimens of 17 cases of head and neck tumors were examined, and the results are summarized in Table 1. Sections of all cases were stained with the antibody to human DCs (RFD1; Fig 1), a mAb that reacts with

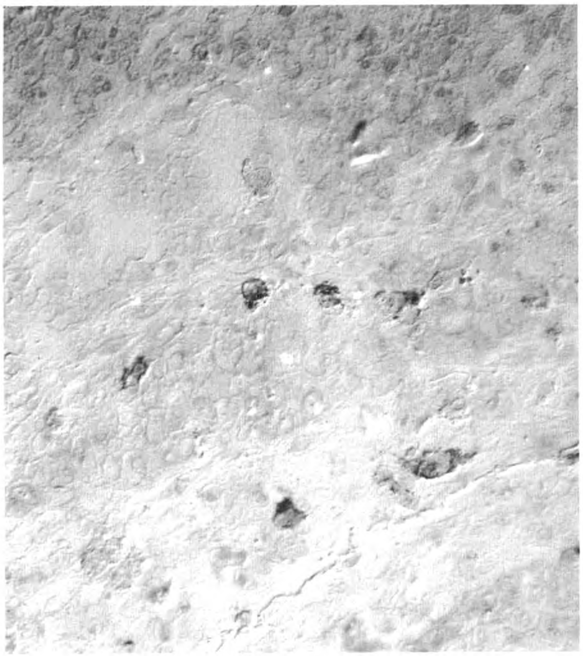

Fig 1. Immunohistochemical visualization of scattered RFD1+ cells (dendritic cells) in epidermoid carcinoma of hypopharynx (original ×400).

mature DCs[21,22] in normal and pathological tissues, and compared with the CD1a (Fig 2) and HLA-DR (Fig 3) mAbs. Positive controls showed immunostaining with these 3 antibodies on tonsil tissue (Fig 4); negative controls showed no immunostaining.

Dendritic cells could be detected in 15 of the 17 cases. They were located between the tumor cells and in the stroma, especially in the areas of inflammatory cell infiltration as defined by abundant staining with anti–T lymphocyte mAbs (see below). In all cases, the infiltration of tumor tissue with DCs was sparse.

In 11 of the 17 cases, we found cells reacting with the mAb RFD1, a marker for mature DCs. Examination of adjacent sections (Fig 5) showed that RFD1+ cells also expressed CD1a and HLA-DR. However, this population was always smaller than the HLA-DR+ or CD1a+ cell populations. Immunoreactive cells were of prominent dendritic shape. In 2 cases, only HLA-DR- and CD1a-expressing, but RFD1-negative, DCs were present. In 2 other cases, DCs expressed CD1a, but not HLA-DR.

Finally, in 2 of the 17 cases, we detected no DCs in the tumor tissue by using the mAbs RFD1 and anti-CD1a. The HLA-DR+ cells in 1 of these cases were identified as activated T cells on the basis of their nondendritic morphology.

In addition, specimens from 9 selected head and neck tumors were immunostained with mAb LAG, which recognizes a cytoplasmic protein associated with Birbeck granules of epidermal LCs.[20] LAG-positive cells were found in 4 of the 9 specimens (Fig 6 and Table 1). Examination of consecutive sections of these 4 specimens revealed that the numbers of LAG-reactive cells were considerably lower than the numbers of HLA-DR+ or CD1a+ cells. Positive controls on tonsillar tissue showed distinct immunostaining of DCs in the epithelium; negative controls were unreactive.

Distribution of Lymphocytes in Tumor Specimens. Specimens of all 17 cases of head and neck tumors were stained with the CD3, CD4, CD8, and CD45RO mAbs (results are summarized in Table 1; interpretations are in Table 2). Positive controls showed immunostaining with these 4 antibodies on tonsil tissue; negative controls showed no immunostaining with the IgG1 and IgG2a mAbs.

T lymphocytes were detected in 16 of the 17 tu-

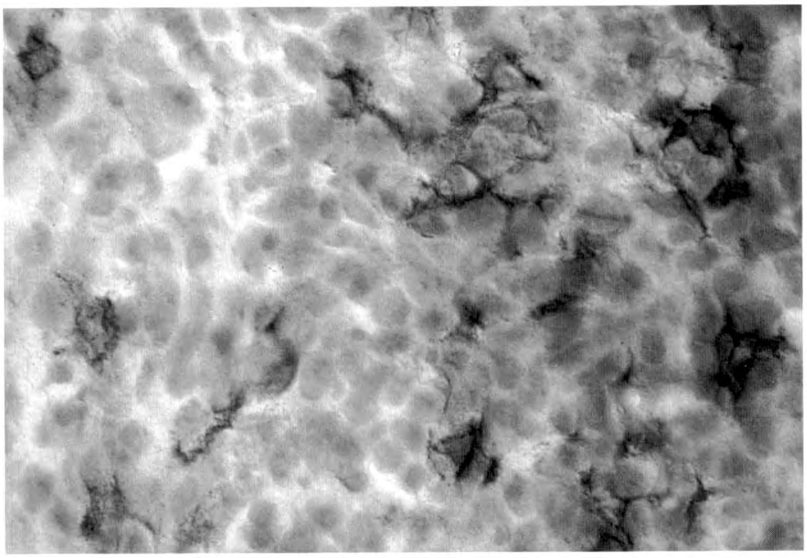

Fig 2. Immunohistochemical visualization of dendritic cells showing typical dendritic morphology of CD1a+ cells in epidermoid carcinoma of paranasal sinus (original ×1,000).

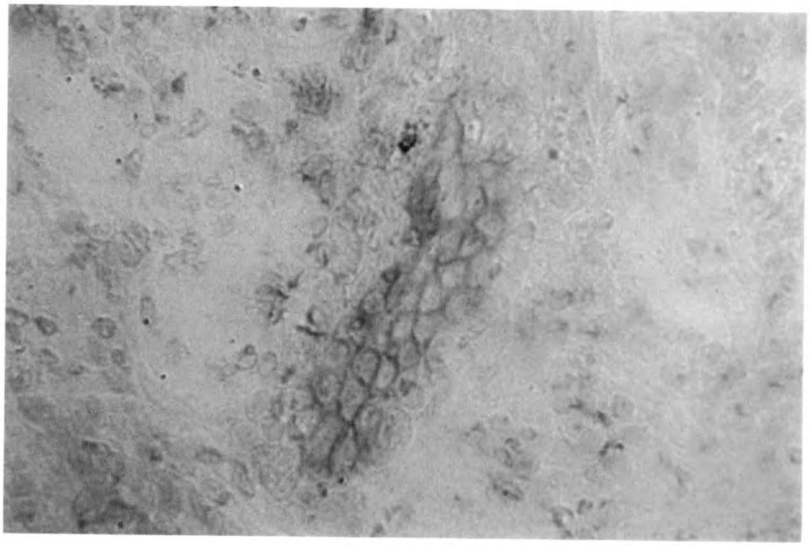

Fig 3. Immunohistochemical visualization of HLA-DR+ cells among tumor cells in adenoid cystic carcinoma of hard palate (original ×400).

mor specimens. The examination of cryostat sections revealed that there was no consistent predominance of either CD4+ or CD8+ T cells. Both types of T cells occurred in variable ratios in the same locations. These ranged from specimens in which only CD3+, CD4+, and CD45RO+ cells (ie, helper T cells only; 1 of 17) were detected to cases containing cells immunostained with all 4 T cell markers (CD3, CD4, CD8, CD45RO; 11 of 17) and cases with the inverse phenotype, ie, CD3+, CD8+, and CD45RO+ cells (ie, cytotoxic T cells only; 2 of 17). In 2 cases, however, we found cells reactive with antibodies against CD3 and CD45RO, but none reactive with antibodies against CD4 and CD8 (possibly γ/δ T cell receptor expressing double-negative T cells). In most cases, the numbers of cells stained with CD45RO were similar to the numbers of CD3+ cells; this finding indicates that most T cells were memory cells.

The examination of cryostat sections also revealed that CD3+ cells were present mainly in the stroma, but also dispersed among tumor cells. The subset of CD4+ helper-inducer T cells was present in aggregates in the stroma. CD8, which identifies the cytotoxic-suppressor T lymphocyte subset, was expressed both on cells present in aggregates in the stroma and among tumor cells.

Distribution of Macrophages in Tumor Specimens. Specimens of all 17 cases of head and neck tumors were stained with the CD68 mAb (results are summarized in Table 1). CD68 is an intracytoplasmic molecule (macrosialin) of monocyte-macrophages and granulocytes.

In all 17 cases, numerous cells reacted positively with the CD68 antibody (Fig 7). Negative controls on tonsil tissue showed no immunostaining with the IgG1 mAb. Positive controls showed immunostaining with CD68. Distribution of CD68+ cells was intraepithelial, stromal, and in the vicinity of tumor cells. They were considerably more numerous than DCs, as defined by HLA-DR and CD1a staining.

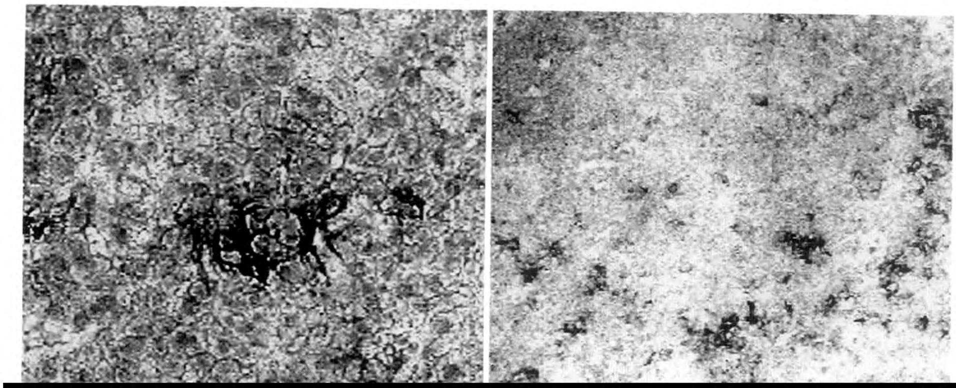

Fig 4. Immunohistochemical visualization of dendritic cells in cryostat sections of normal human tonsils. A) Scattered cells reactive with monoclonal antibody against CD1a (original ×200). Note typical dendritic processes. B) Monoclonal antibody RFD1 labels dendritic cell profiles in

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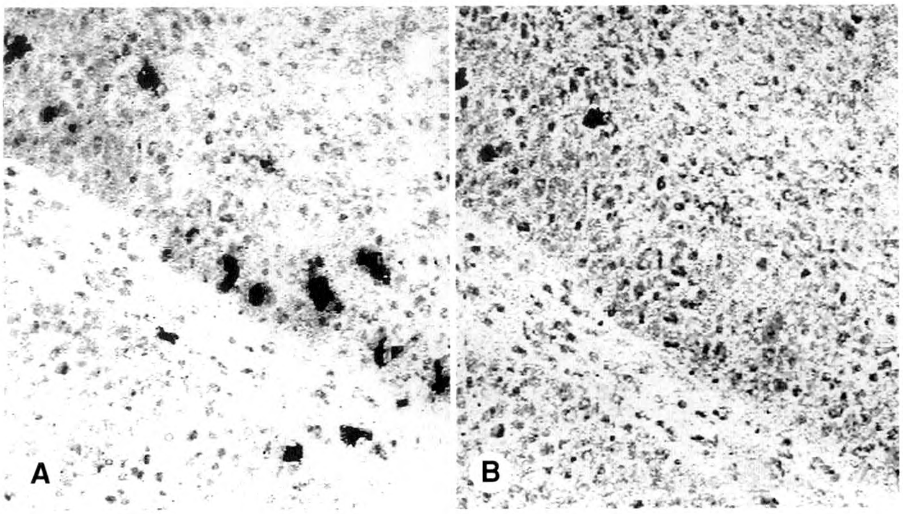

Fig 5. Immunohistochemical visualization of dendritic cells in adjacent sections of same area of tongue carcinoma (original ×100). A) Scattered cells reactive with monoclonal antibody against CD1a. B) Monoclonal antibody RFD1 labels only 3 of these dendritic cells.

DISCUSSION

Several reports have been published concerning the morphology and distribution of tumor DCs.[16,23-27] Here we show that DCs can be readily identified in head and neck tumors by immunohistochemistry. The cells were identified by their expression of CD1a and HLA-DR. These cells had typical dendrites extending between neighboring cells. They were located in the stroma and also, more importantly, in the tumor tissue. Phenotyping of these cells revealed that they were heterogeneous in nature. The subset of DCs expressing the molecule recognized by mAb RFD1 represents mature DCs. RFD1 expression has been described on mature cutaneous DCs, such as dermal DCs[10] and cultured epidermal LCs, but not on immature resident LCs.[22] Even fewer cells were reactive with mAb LAG, which recognizes Birbeck granules of LCs.[20] This finding may indicate that most tumor-infiltrating DCs are not epidermal LCs in transit, but rather, that they constitute a separate population of tumor-resident DCs.

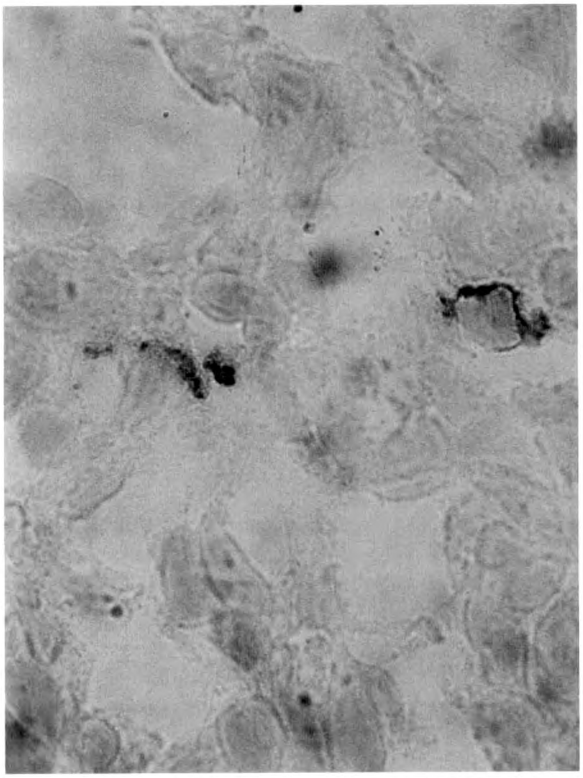

Fig 6. Immunohistochemical visualization of LAG$^+$ Langerhans cells in epidermoid epiglottic carcinoma showing Birbeck granules (original ×1,000).

The distribution and/or morphology of DCs also differed from that of macrophages, resting T cells, and endothelial cells. Interestingly, the tumors were infiltrated by considerable numbers of CD68$^+$ macrophages. The CD68$^+$ macrophages were distributed intraepithelially, stromally, and in the vicinity of tumor cells and were considerably more numerous than DCs. The presence of this cell type has previously been correlated with an improved prognosis for patients.[27] One may speculate that the tumoricidal mech-

TABLE 2. REACTIVITY OF HEAD AND NECK TUMORS WITH MONOCLONAL ANTIBODIES IDENTIFYING T CELLS

No. of Cases	*CD3*	*CD4*	*CD8*	*CD45RO*	*Interpretation*
11	+	+	+	+	Helper and cytotoxic (mostly memory) T cells
1	+	+	–	+	Helper T cells only (mostly memory)
2	+	–	+	+	Cytotoxic T cells only (mostly memory)
2	+	–	–	+	Possibly γ/δ T cell receptor expressing double-negative (mostly memory) T cells
1	–	–	–	–	No T cells

+ — Presence of immunoreactive cells; – — absence of immunoreactive cells.

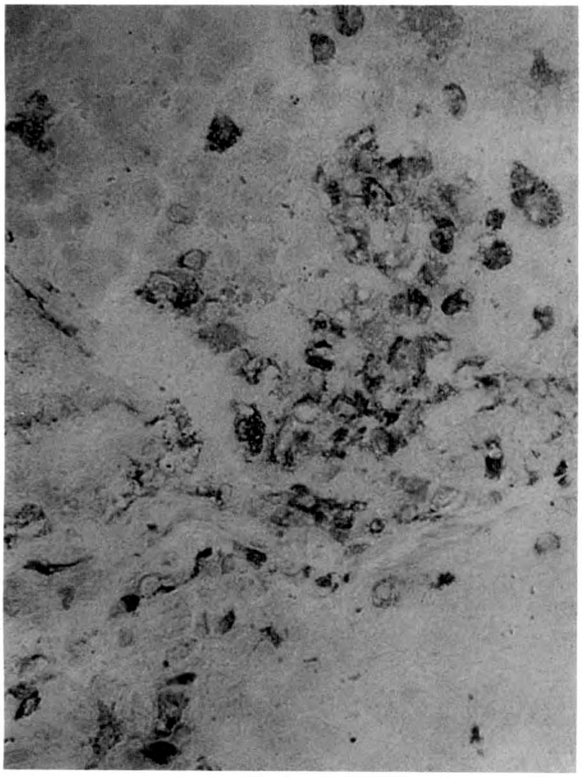

Fig 7. Immunohistochemical visualization of macrophages in adenocarcinoma of paranasal sinus (original ×400). $CD68^+$ cells (macrophages) are visible in stroma and in vicinity of tumor cells.

anisms of macrophages contribute to this phenomenon.

T-helper-inducer cells ($CD4^+$) were present in aggregates in the stroma; T-cytotoxic-suppressor cells ($CD8^+$) were present both in aggregates in the stroma and among tumor cells. In the head and neck tumors, there was no consistent predominance of either $CD4^+$ or $CD8^+$ T cells. Both types of T cells occurred in variable ratios in the same locations. In most cases, the number of $CD45RO^+$ cells was similar to the number of $CD3^+$ cells, indicating that most T cells were memory cells.

We agree with others[4,17] that a dense infiltration with DCs is rarely present in malignant lesions of the head and neck. This paucity of DCs may lead to insufficient stimulation of cytotoxic T lymphocytes and, therefore, to an insufficient antitumor immune response. It has been demonstrated that tumor-associated DCs are deficient in important T cell costimulatory molecules.[16] This may explain why they fail to induce an efficient cytotoxic response to eradicate tumor cells.

It is known that peritumoral $CD1a^+$ DCs are associated with improved survival in patients with tongue carcinoma[28] and that nasopharynx carcinoma patients that have a dense tumor infiltration of DCs survive longer and have a better prognosis.[29,30] The prognosis is also more favorable when a T cell infiltration is associated with the infiltration of antigen-presenting cells.

Recent studies have shown the therapeutic application of the antitumor properties of bone marrow–derived DCs.[14,31-33] The prerequisites for the possible success of such an antitumor therapy have been recently fulfilled. They include the generation of fully mature DCs in sufficient quantities[34] and the identification of tumor antigens, which are now being cloned in increasing numbers.[15] These tumor antigens have also been detected in head and neck tumors.[35-37] With regard to the use of DCs in tumor therapy, optimism is justified[13]; precocious enthusiasm, however, is not warranted, because too many problems concerning this process are yet to be solved.

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GIANT CELL TUMOR OF THE LARYNX

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Giant cell tumors are benign tumors generally found in the long bones. Very rarely, they can occur in the larynx and may present with dysphonia, dysphagia, or dyspnea. A case of giant cell tumor of the larynx was recently identified and successfully treated by a partial laryngectomy. A literature review has revealed 18 case reports of giant cell tumor of the larynx. All cases occurred in men. These 19 cases are reviewed, and follow-up data presented where available. There have been no reports of recurrence regardless of treatment, and an excellent prognosis can be expected when one encounters this unusual laryngeal neoplasm.

KEY WORDS — benign laryngeal neoplasms, dysphonia, dyspnea, giant cell tumor.

INTRODUCTION

The frequency of primary supporting tissue neoplasms in the larynx is approximately 2% of all primary laryngeal neoplasms.[1] The majority of these neoplasms are cartilaginous tumors, with fibrosarcomas, hemangiomas, and other nonepithelial tumors of the larynx being somewhat less common. Among tumors and tumorlike conditions reported to involve the osteocartilaginous structures of the larynx are osteosarcoma, aneurysmal bone cyst, chondrosarcoma, and chondroma.

Giant cell tumor is a benign neoplasm occurring most commonly in the long bones. It accounts for approximately 5% of all primary bone neoplasms and is potentially aggressive. Rarely, it is identified in the skull or cervical spine, and tumors here account for no more than 4% of all giant cell tumors. When there is skull involvement, the temporal, sphenoid, and ethmoid bones are most commonly affected. Recently, a giant cell tumor of the larynx was diagnosed and treated, stimulating a review of the subject. A literature review revealed 18 similar cases. These case reports and follow-up data (where available) will be reported together with a discussion of the case at hand and a review of our own pathology registry.

CASE REPORT

A 31-year-old man presented with a 2-month history of hoarseness and a lump in the neck. His hoarseness came on gradually over a 1- to 2-week period and steadily increased in severity. Additionally, a firm lump in the left side of the neck was noted. The patient denied dyspnea, dysphagia, odynophagia, or hemoptysis. The lump in the left side of the neck was completely asymptomatic, except that it had slowly enlarged over the past 2 months. The patient has no prior history of head and neck disease and is otherwise healthy. He is a nonsmoker and nondrinker.

Physical examination revealed normal vital signs in an otherwise healthy-appearing young man. His voice was hoarse but easily understandable. Examination of the external auditory canals and tympanic membranes, nose, mouth, nasopharynx, oropharynx, and hypopharynx revealed no abnormalities. Examination of the larynx revealed a smooth, large, mucosa-covered, nonulcerated intralaryngeal mass limited to the left hemilarynx, displacing the true and false vocal folds medially and extending superiorly into the aryepiglottic fold. Palpation of the neck revealed a firm 4-cm smooth mass fixed to the left hemilarynx. The larynx was mobile over the pharynx and prevertebral structures, with normal excursion during swallowing. There was no identifiable lymphadenopathy in the neck.

Laboratory evaluation included a fine needle aspiration and a high-resolution computed tomography (CT) scan of the neck. The needle aspiration revealed numerous multinucleated giant cells. The CT scan of the neck revealed a homogeneous neoplasm arising from the left thyroid cartilage, seemingly exploding it from within (see Figure, A). The mass extended inferiorly to involve the left anterolateral cricoid cartilage.

A presumptive diagnosis of giant cell tumor of bone within the larynx was made, and the patient was counseled about treatment options, including surgery and radiotherapy. The patient subsequently underwent tracheostomy, cervical exploration, and excision of the neoplasm. A vertical hemilaryngectomy, including the involved portion of cricoid cartilage, was necessary. Histologically clear margins were seen on frozen section pathology.

The patient was discharged from the hospital on

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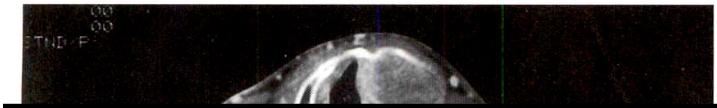

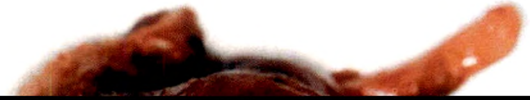

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SUMMARY OF REPORTED CASES OF GIANT CELL TUMOR OF LARYNX

Authors	*Year*	*Age*	*Location*	*Treatment*	*Follow-up*
Wessely[4]	1940	51	Cricoid	Irradiation	
Federova[5]	1951	35	Thyroid	Hemilaryngectomy	
Wagemann[6]	1952	40	Cricothyroid	Irradiation and laryngectomy	NED 7 y
Perrino[7]	1958	32	Cricoid	Laryngofissure and irradiation	
Kaliteevskii and Korol'kova[8]	1966	52	Thyroid	Laryngectomy	
Pohl[9]	1968	50	Thyroid	Irradiation	NED 2 y
Kohn[10]	1969	50	Epiglottis	Unknown	
Rudert[3]	1971	53	Thyroid	Hemilaryngectomy	NED 8 y
Hall-Jones[11]	1972	26	Thyroid	Laryngectomy	NED 18 mo
Goto and Nakashima[12]	1973	47	Thyroid	Partial laryngectomy	
Kotarba and Niezabitowski[13]	1974	60	Epiglottis	Hemilaryngectomy	NED 6 mo
Ribari et al[14]	1975	35	Cricoid	Laryngectomy	NED 72 mo
Kubo et al[15]	1976	40	Cricoid	Laryngofissure, 5-fluorouracil, and irradiation	
Tsybyrne and Bogdanskaia[16]	1979	34	Thyroid	Laryngofissure and irradiation	
Borghese et al[17]	1988	28	Thyroid	Laryngofissure	NED 15 mo
Murrell and Lantz[18]	1993	42	Thyroid	Laryngectomy	
Martin et al[19]	1994	23	Thyroid	Hemilaryngectomy	NED 5 y
Werner et al[2]	1997	35	Thyroid	Laryngectomy	NED 7 1/2 y
Present case		31	Thyroid	Hemilaryngectomy	NED 3 y

NED — no evidence of disease.

resented a metastasis from a femoral primary and was not included. This leaves a total of 19 cases, including the present one (see Table[2-19]). All tumors occurred in men between the ages of 23 and 60. When reported, the average diameter of the tumor measured 4.5 cm, with the cricoid and thyroid cartilages being the most common locations. Treatment and follow-up data, where available, are also given in the Table. Eleven patients were treated with surgery alone, 2 patients were treated with irradiation alone, and 5 patients were treated with combined surgery and irradiation. The treatment utilized in the remaining patient was not reported. Follow-up data beyond 2 years are available on only 7 patients. However, there have been no reports of recurrence, regardless of the therapy chosen, even when histologically positive margins remained.

Giant cell tumors are rare lesions that constitute roughly 4% of all primary bone tumors. The vast majority of these tumors occur in the epiphysis of the long bones. Approximately 2% of all giant cell tumors occur in the head and neck region. Most of these occur within the sphenoid, ethmoid, or temporal bones.[20]

The common head and neck sites for these tumors correspond to the cranial bones formed by endochondral ossification. The laryngeal skeleton is primarily composed of hyaline cartilage until approximately the second decade of life, at which time it may gradually begin to be replaced by bone. This occurs at different rates in different individuals, but occurs earlier in males and includes endochondral ossification with marrow spaces in sites of hematopoiesis. All reported cases of giant cell tumor in the larynx have occurred in men over the age of 20. Interestingly, giant cell tumors of the long bones are more common in females, with a ratio of 3 to 2.[21] A relationship between the maturation process of the larynx of men and the development of giant cell tumors in this location is unclear but plausible. Our case, like that of Martin et al,[19] was rimmed by osseous tissue. This suggests that the tumor began in the marrow space between cortical layers. It is clear from the literature on long bone tumors that a marrow space is a prerequisite to the development of these tumors.[22]

Most investigators conclude that the progenitor cell in giant cell tumors is a primitive mesenchymal cell found in bone marrow. These primitive cells may remain dormant for unspecified periods of time until an unknown stimulus induces metaplastic change and proliferation. No inducer of this change has been identified.[19] The lesion is a true neoplasm, probably best included in the fibrohistiocytic series.[23]

Both surgery and radiotherapy appear to be viable treatment options. The issue of sarcomatous transformation following irradiation to giant cell tumors is evident from the orthopedic literature and has been reported to be as high as 25%.[24] This figure has been blamed on older orthovoltage radiation equipment, and in 1 recent study of 15 long bone tumors treated with newer supervoltage radiotherapy, there were no recurrences.[25] Larger series are needed to help document the effectiveness and safety of radiotherapy. The incidence of primary malignancy in the head and neck following irradiation is quite low (0.2%), but this might also be considered an issue, as all tumors have occurred in young patients.[26,27] The treating physician must decide if these numbers are important when treating a giant cell tumor of this type. On the basis of those few cases reported, good results with partial laryngeal surgery can be expected.

In conclusion, I report an additional case of giant cell tumor of the larynx treated by surgery alone. Follow-up over 3 years reveals no evidence of recurrence. A review of the literature revealed 18 additional reports. All tumors occurred in young men. One can expect an excellent prognosis when treating these tumors.

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MORPHOMETRIC AND HISTOCHEMICAL STUDY OF THE HUMAN VOCAL MUSCLE

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The present study was conducted on vocal muscles removed at autopsy from adult individuals (10 men and 8 women, ages ranging from 48 to 78 years) with no laryngeal disease. Histologic analysis was performed with hematoxylin and eosin staining, and histochemical analysis was performed by nicotinamide-adenine-dinucleotide tetrazolium reductase, succinate dehydrogenase, and acid and alkaline myofibrillar adenosine triphosphatase reactions. The histochemical reactions showed that the muscle consists of slow-twitch oxidative (SO), fast-twitch glycolytic (FG), and fast-twitch glycolytic oxidative (FOG) fibers distributed in mosaic form. The frequencies of SO, FOG, and FG fibers were 40.50%, 54.75%, and 4.75%, respectively. The higher frequency of SO and FOG oxidative fibers characterizes the muscle as having aerobic metabolism, resistance to fatigue, and fast contraction. The mean minimum diameters were 31.37 µm for SO fibers and 36.46 µm for FOG and FG fibers.

KEY WORDS — human, larynx, morphology, vocal muscle.

INTRODUCTION

The major functions of the larynx are to permit a free passage of air in order to promote gas exchange and to prevent the entry of foreign bodies into the lower airways. With species evolution, the need for communication arose and humans learned to prolong expiration and to coordinate it with glottic adduction, thus promoting a vibratory cycle of the vocal folds that resulted in voice production.

The anatomy of the vocal folds has been extensively studied, especially after Husson[1] proposed in his neuromuscular theory that the vocal folds do not vibrate solely under the influence of a subglottic air current, but also actively vibrate by simultaneous and rhythmic contraction of the vocal muscle in response to successive stimuli from the central nervous system via the recurrent laryngeal nerve.

Muscles have been classified in different ways on the basis of biochemical studies, taking into account their metabolic activity, contraction velocity, and fatigability of muscle fibers.[2-4] The objective of the present study was to analyze the histologic, histochemical, and morphometric aspects of the vocal muscle in adults of different ages, and to provide further information about the relationships between the structural and metabolic characteristics of the muscle and its functional properties.

MATERIAL AND METHODS

The vocal muscles to be submitted to histologic and histochemical analyses were removed at autopsy within 6 hours of death from 18 adult cadavers (10 men and 8 women) with ages ranging from 48 to 78 years. Fragments of each muscle were immersed in liquid nitrogen at −160°C, transferred to a cryostat chamber at −20°C, and cut into 10-µm-thick serial sections. The sections were stained with hematoxylin and eosin (H & E) and submitted to the following reactions: the nicotinamide-adenine-dinucleotide tetrazolium reductase (NADH-TR) method as described by Pearse,[5] the succinate dehydrogenase (SDH) method of Wegman and Tordet-Caridroit,[6] and the myofibrillar adenosine triphosphatase (mATPase) method at pH 10.2 (alkaline) and 4.6 (acid) as described by Brooke and Kaiser.[2]

The material was analyzed in terms of histology, the proportions of different muscle fiber types, and the minimum fiber diameter. Measurements were made in the middle area of the vocal muscle (500 fibers for each muscle). Fiber diameter was determined by measuring the maximum distance across the smaller dimensions of the muscle fiber.

Morphometric data were obtained with the Bioscan-Optimas 4.1 image analysis computer system coupled to a light microscope with a 16× objective.

RESULTS

Transverse histologic sections stained with H & E showed each muscle fiber of the vocal muscle, surrounded by a well-developed layer of connective tis-

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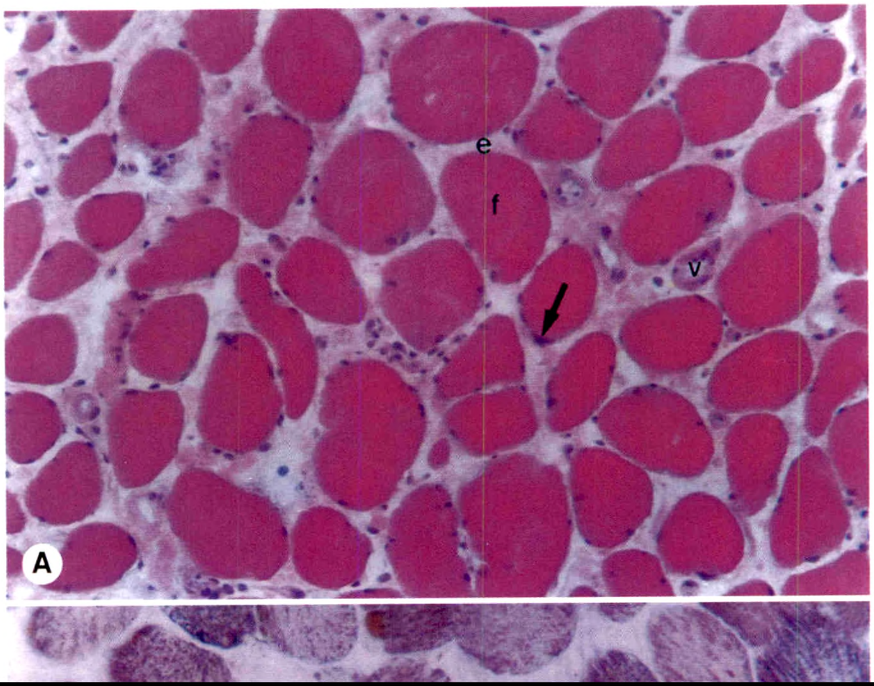

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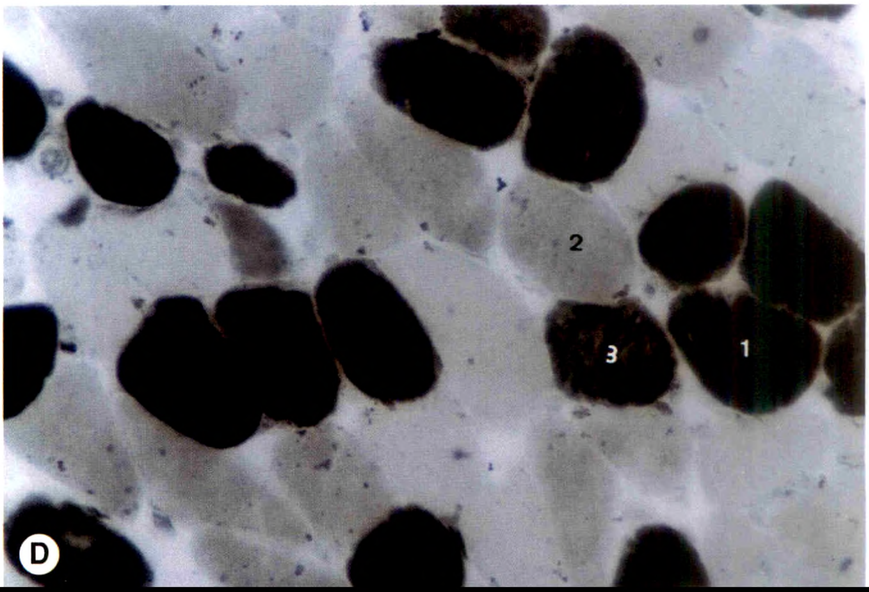

(Continued.) D) Submitted to acid mATPase reaction, classifying muscle fiber types SO (1), FOG (2), and FG (3).

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TABLE 1. ENZYMATIC REACTIVITY OF SO, FOG, AND FG MUSCLE FIBERS IN HUMAN VOCAL MUSCLE

	Enzymes			
Fibers	*mATPase, pH 10.2*	*mATPase, pH 4.6*	*NADH-TR*	*SDH*
SO	+	+++	+++	+++
FOG	+++	+	++	++
FG	+++	++	+	+

SO — slow-twitch oxidative; FOG — fast-twitch glycolytic oxidative; FG — fast-twitch glycolytic; mATPase — myofibrillar adenosine triphosphatase; NADH-TR — nicotinamide-adenine-dinucleotide tetrazolium reductase; SDH — succinate dehydrogenase; +++ — strong reaction; ++ — intermediate reaction; + — weak reaction.

were $t = 0.280$ for SO ($p > .50$) and $t = 1.322$ for FOG and FG ($p < .20$). Thus, the right and left sides presented similar SO and FOG and FG values for the 2 sides.

The results of the *t*-test for the comparison of mean minimum diameter between SO and FOG and FG fibers on both sides were right side, $t = 3.65$ for SO versus FOG and FG ($p < .01$); left side, $t = 3.993$ for SO versus FOG and FG ($p < .001$). Thus, the minimum diameter of SO fibers was smaller than that of FOG and FG fibers on both sides.

The results showed that a significant correlation (Pearson correlation coefficient) was not found between the ages and the percentages of the fiber types, nor between the ages and the minimum diameters of the fiber types.

DISCUSSION

The vocal muscle is the structure that forms the body of the vocal fold.[7] In the present study, transverse sections of the muscle stained with H & E showed muscle fibers of a circular shape, multinucleated and with peripheral nuclei, that were surrounded by a well-developed connective tissue layer, the endomysium. Other investigators have also observed these histologic characteristics and have pointed out the difference in shape of the vocal muscle compared to the skeletal muscles of the limbs, which are polygonal in shape.[8,9]

The histochemical analyses revealed the presence of 3 types of muscle fibers classified as follows according to their oxidative or glycolytic metabolic activity and according to contraction time[3]: FG, SO, and FOG.

TABLE 2. PERCENTAGE OF SO, FOG, AND FG FIBER TYPES IN HUMAN VOCAL MUSCLE ACCORDING TO SIDE

	Right Side (%)				*Left Side (%)*			
	SO	*FOG*	*FG*	*FOG and FG*	*SO*	*FOG*	*FG*	*FOG and FG*
Mean	40	55.5	4.5	60	41	54	5	59
SD	7.68	9.67	3.31	7.68	9.95	11.1	3.56	9.95

TABLE 3. MINIMUM DIAMETER OF SLOW-TWITCH AND FAST-TWITCH MUSCLE FIBERS IN HUMAN VOCAL MUSCLE

	Right Side (μm)		*Left Side (μm)*	
	SO	*FOG and FG*	*SO*	*FOG and FG*
Mean	31.50	35.69	31.25	37.24
SD	6.44	4.78	6.42	7.62

Muscle fiber typing was performed with the NADH-TR and SDH reactions, while the type of contraction was determined with the mATPase reaction at pH 10.2 and with the mATPase reaction after preincubation at acid pH. The latter determination is based on the fact that myosin is stable at acid pH and labile at alkaline pH in slow-twitch fibers, and myosin is labile at acid pH and stable at alkaline pH in fast-twitch fibers.[10]

The mATPase reaction at alkaline pH revealed SO muscle fibers and FOG and FG fibers in the human vocal muscle. Other investigators[8,9,11,12] also detected 2 types of muscle fibers using the mATPase reaction at alkaline pH and classified slow-twitch and fast-twitch fibers as type I and type II, respectively.

However, when acid mATPase was used, type II fast-twitch fibers were subdivided into IIA and IIB,[2] corresponding to the FOG and FG fibers detected in the present study. This differentiation is made possible by the fact that FOG fibers lose their ATPase activity after acid preincubation at pH 4.6, while FG fibers continue to be slightly active.[13,14]

The frequency of SO muscle fibers in our study was 41% and the frequency of FOG and FG fibers was 59%. The literature also reports higher percentages of fast-twitch fibers.[9,11-13] However, some investigators[15] found equal percentages of slow- and fast-twitch fibers. Further, slightly higher percentages (53%) of slow-twitch fibers have been reported.[8,14]

There is a good correlation between contraction velocity and mATPase activity in muscle fibers.[4] Thus, the predominance of FOG and FG fibers detected here corresponds to the data obtained in physiological studies of the laryngeal muscle of dogs, primates, and rabbits[16-18] that have classified the vocal muscle as a fast-twitch muscle.

Teig et al[11] attributed fast contraction properties to the human vocal muscle after comparing it to other fast-twitch muscles, among them the tensor tympani muscle of the cat, which presented 40% SO fibers and 60% FOG and FG fibers.

Analysis of muscle fiber counts revealed that the human vocal muscle has a predominantly aerobic oxidative metabolism, and is therefore a muscle resistant to fatigue. This result agrees with findings reported by other investigators,[13,14] who also found a predominance of SO and FOG fibers in the vocal muscle.

The distribution of SO, FOG, and FG muscle fibers presented a typical mosaic pattern, although there were also small areas of fibers of the same group clustered together, reflecting the appearance of metabolic groups.[9]

The minimum diameter of the muscle fibers was measured in transverse sections of the vocal muscle, since this leads to a lower probability of variation in the results in case some fibers sectioned obliquely or lengthwise should be present during analysis.[19]

The minimum diameters of the human vocal muscle fibers were on average 31.37 μm for slow fibers (SO) and 36.46 μm for fast fibers (FOG and FG). Other investigators have also observed that fast muscle fibers present, on average, a wider diameter than slow fibers.[9,13,20]

No significant variation in minimum fiber diameter was detected with respect to age. In addition, the minimum diameter values obtained for SO, FOG, and FG fibers did not vary from the right to the left side. These data agree with those reported by Rodeño et al.[20]

We conclude that the vocal muscle has a predominance of muscle fibers of oxidative metabolism, which render it resistant to fatigue — a finding of great clinical importance because of the continuous action of this muscle during phonation. With respect to contraction velocity, the vocal muscle proved to be a rapid muscle with a predominance of FOG and FG fibers in relation to SO fibers, a property that permits it to perform fast movements during brusque tonal variations in voice and during the sudden sphincteric action of the protective reflex.

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SPONTANEOUS RESPIRATION ANESTHESIA FOR RESPIRATORY PAPILLOMATOSIS

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Several anesthetic techniques for microlaryngeal laser surgery in children have been advocated. The objective of this study was to evaluate the operative conditions and safety of the spontaneous respiration anesthesia technique for carbon dioxide laser surgery in children with recurrent respiratory papillomatosis (RRP). The anesthetic and operative records of 60 pediatric patients with RRP who underwent microlaryngeal laser surgeries between 1991 and 1997 were reviewed. Papilloma location, severity of involvement, oxygen saturation, airway stability, need for intubation, duration of procedure, and complications were recorded. The patients underwent 744 surgical procedures. Two hundred two procedures were randomly studied (statistical power ≥ 80%). The supraglottis and glottis were most commonly involved. Most of the papillomas were graded as moderate or severe disease. Complications occurred in 11 procedures. The surgical procedure was completed in all cases. No variable had predictive value regarding the outcome. Spontaneous respiration anesthesia is an efficient and relatively safe technique for microlaryngeal laser surgery in children with RRP.

KEY WORDS — anesthesia, children, laser, respiratory papilloma.

INTRODUCTION

Suspension microlaryngoscopy with carbon dioxide (CO_2) laser ablation is the procedure of choice for treatment of recurrent respiratory papillomatosis (RRP).[1] The goal of laser therapy is the safe removal of papilloma from the airway with minimal trauma to surrounding normal tissue. One of the most important factors in achieving this goal is the maintenance of adequate anesthesia during the procedure. Laser surgical procedures in infants and children with RRP present special problems owing to the small diameter of the pediatric airway, which is aggravated by the exophytic growth of the papilloma.

The fact that children with RRP often require surgery at frequent intervals increases the need for an efficient and safe anesthetic technique. The ideal anesthetic technique would provide complete unimpeded access to the larynx with no motion of the vocal cords, maintaining a safe airway with adequate ventilation and permitting a rapid emergence from anesthesia. In addition, the application of CO_2 laser energy requires a technique that minimizes the risk of combustion. Several anesthetic techniques for microlaryngeal laser surgery have been advocated. These techniques include ventilation through a "laser-safe" endotracheal tube (ETT), jet ventilation, apneic anesthesia, and spontaneous respiration anesthesia (SRA). However, controversy exists regarding the best anesthetic technique. In a recent national survey regarding the management of RRP in adult and pediatric patients, 46% of the respondents favored the use of laser-safe ETTs, 26% favored jet ventilation, 16% preferred using an apneic technique, and only 12% preferred using SRA.[2]

In children undergoing laser microlaryngeal surgery for RRP, SRA has been used as the main anesthetic technique at the Children's Hospital Medical Center in Cincinnati since 1986. The SRA technique used in our institution is a modification of the technique first described for laryngeal microsurgery by Judelman[3] and Talmage.[4] Spargo et al[5] advocated this technique for children undergoing CO_2 laser treatment of RRP. However, their recommendation was based on only 63 procedures, and the outcome measurements were not clearly defined.

The purpose of this report is to describe our experience with this technique in the treatment of children with juvenile RRP and to draw attention to its potential advantages.

SUBJECTS AND METHODS

From January 1, 1991, to December 31, 1996, 60

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TABLE 1. PAPILLOMA STAGING

Stage	*Definition*
1	Minimal papilloma occupying less than 10% of anatomic site
2	Moderate papilloma occupying 10% to 50% of anatomic site
3	Severe papilloma occupying more than 50% of anatomic site

pediatric patients (18 years of age and under) with RRP underwent 744 laser microlaryngeal surgeries in the Department of Pediatric Otorhinolaryngology at Children's Hospital Medical Center, Cincinnati, Ohio. Operative procedures were evaluated retrospectively. Patients with tracheotomy tubes were excluded from this study. A review of the anesthetic and operative records was performed for each procedure. Each procedure was scored with respect to patient age, weight, gender, American Society of Anesthesiologists (ASA) classification, sites of papilloma involvement (supraglottis, glottis, subglottis, trachea, bronchi), severity of papilloma involvement, anesthetic agents used for induction and maintenance of anesthesia, oxygen (O_2) flow, and duration of the procedure. The papilloma in each site was staged from 1 to 3 (Table 1). The SRA technique was determined to be a failure if any of the following occurred: intubation during the procedure, arterial desaturation of <92%, bradycardia, aspiration, inadequate air exchange, laryngospasm, inadequate depth of anesthesia, or abandonment of the technique for any other reason. Elective intubation at the end of the procedure for emergence was not considered a failure. In addition, all records were reviewed for the incidence of other complications such as arrhythmia, hypertension, hypotension, or difficulties during emergence.

Anesthetic Technique. Anesthetic and airway management techniques are discussed with the parents and patient by the attending anesthesiologist and otolaryngologist as part of every preoperative evaluation. Since frequent, repeated anesthetics are often required for children with RRP, every effort is made to reduce the stress experienced by the child. Parents are allowed to accompany their child to the induction room and to be present during induction of anesthesia.

Anesthesia was induced either by inhalation agents (halothane, sevoflurane) or intravenous agents (propofol, thiopental sodium). The choice of induction agent was determined by the anesthesiologist and patient preference. Most children who received an intravenous induction had chosen to do so. Once an adequate depth of anesthesia to permit laryngoscopy was achieved, the larynx was topically sprayed with 4% lidocaine hydrochloride topical solution (≤5 mg/kg). Maintenance of anesthesia was accomplished by inhalation agents (halothane, isoflurane, sevoflurane), intravenous propofol, or a combination of inhalation gases and propofol. After the depth of anesthesia was felt to be adequate, suspension laryngoscopy was performed. An appropriate laryngoscope was positioned in the airway coupled to a Cotton-Johnson modified Tobey laryngoscope holder (Pilling-Weck, Research Triangle Park, NC) supported by a plastic table (Fig 1). Until 1995, anesthetic gases were delivered through an appropriately sized Frazer suction tip with a 15-mm anesthetic adaptor,[6] or through a Johnson adaptor,[7] both of which were placed in the left channel of the laryngoscope. Since 1995, anesthetic gases have been delivered through a left side arm incorporated into the laryngoscope (Pilling-Weck; Fig 2). The total flow of gases is 5 to

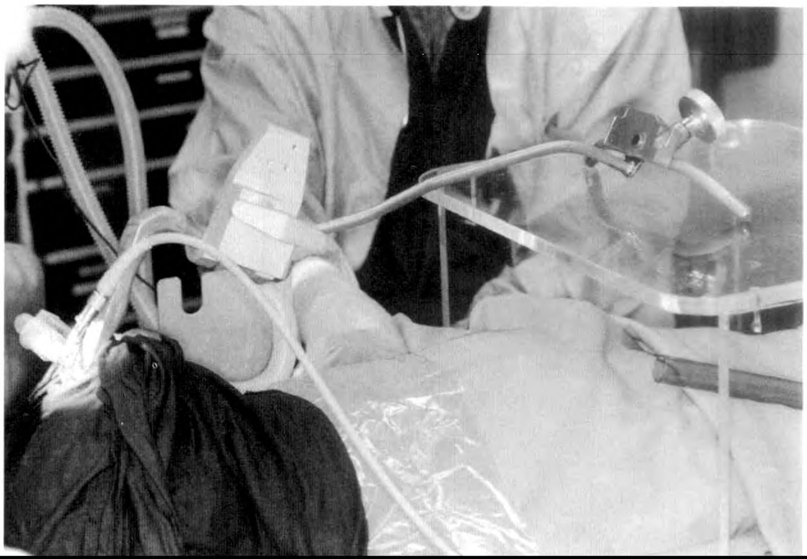

Fig 1. Cotton-Johnson modified Tobey laryngoscope holder (Pilling-Weck, Research Triangle Park, NC) supported by plastic table.

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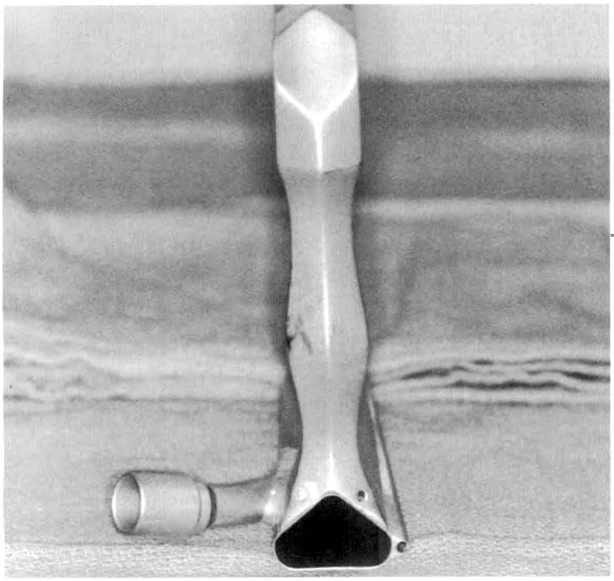

Fig 2. Cotton-Jacob laryngoscope (Pilling-Weck) with left side arm for delivery of anesthesia gas.

6 L/min. Fairly high concentrations of anesthetic gases are required due to the open nature of the system, in which room air may be entrained by the patient. Spontaneous ventilation is required for delivery of inhaled anesthetic gases. However, if necessary, controlled ventilation can be accomplished by placing a gloved thumb over the port of the suspension laryngoscope. The appropriate model and size of the laryngoscope is selected according to the site of disease and patient age. The patient's eyes and face are protected with moistened eye pads and 2 layers of moistened towels (Fig 2). A Zeiss microscope (Carl Zeiss, Oberkochen, Germany) with a CO_2 laser attachment (Sharplan, Israel) is then brought into position. A large suction evacuator is placed near the laryngoscope port to evacuate smoke plume and anesthetic gases. Brief elective intubation

[redacted]

TABLE 2. PAPILLOMA STAGING PERFORMED DURING MICROLARYNGEAL PROCEDURE

	Stage			
Site	*1*	*2*	*3*	*Total*
Supraglottis	46	91	37	174
Glottis	59	102	15	176
Subglottis	31	11		42
Trachea	9			9

tion rate. Statistical power of ≥80% was considered sufficient to represent the entire number of procedures. The Kendall correlation coefficients procedure was used to calculate simple associations between the variables (age, weight, gender, ASA classification, disease sites, severity of disease, anesthetic agents, and duration of procedure). A logistic regression procedure was used to calculate the correlation between the measured variables and the outcome (complication). Since the duration of procedure could be a result of complications occurring during the procedure, a stepwise logistic regression procedure with exclusion of the duration of the procedure was used to evaluate the independent predictive value of the variables regarding the outcome.

RESULTS

Two hundred two procedures were randomly reviewed. By using the hypergeometric model (in consideration with the rate of complications noted in this series), a statistical power of >80% was calculated. Therefore, no further accumulation of data was necessary. The patients ranged in age from 9 months to 18 years (mean ± SD, 5.6 ± 4.1 years), and in weight from 8 to 55 kg (mean ± SD, 22.66 ± 10.8 kg). The patients were classified as having stage 2 disease in 171 procedures, stage 3 in 18 procedures, and stage 1 in 13 procedures. The supraglottis was involved

TABLE 3. INDUCTION AND MAINTENANCE AGENTS (N = 202)

	Agent	*Procedures* *No.*	*%*
Induction	Halothane	113	56
	Propofol	63	31.2
	Sevoflurane	25	12.4
	Thiopental sodium	1	0.5
Maintenance	Halothane	127	62.9
	Halothane-propofol	56	27.7
	Isoflurane	5	2.5
	Isoflurane-propofol	4	2.0
	Sevoflurane	4	2.0
	Propofol	3	1.5
	Sevoflurane-propofol	2	1.0

TABLE 4. COMPLICATIONS (N = 202)

Complication	*Procedures* *No.*	*%*
Arterial desaturation	3	1.5
Laryngospasm	3	1.5
Airway obstruction	2	1.0
Coughing	1	0.5
Vomiting	1	0.5
Arrhythmia	1	0.5
Total	11	5.5

tients had arterial desaturations during the procedure. Two of these patients were intermittently intubated. The third patient maintained O2 saturations in the 88% to 92% range and did not require intraoperative intubation. Three patients experienced laryngospasm; in 2 of these, it was during induction, and the patients were then paralyzed and intubated. The third patient had laryngospasm during the procedure and required paralysis and intermittent intubation for the remainder of the procedure. Two patients had significant airway obstruction during the procedure and required intermittent intubation with an ETT passed through the suspension laryngoscope. One patient coughed during the procedure due to inadequate depth of anesthesia; an ETT tube was inserted to allow an increased delivery rate of anesthetic gas. One patient vomited during induction. This patient was paralyzed and intubated and had a nasogastric tube inserted. No aspiration occurred, and the procedure was completed with intermittent intubation. One patient experienced a brief period of bigeminy immediately after inhalation induction with sevoflurane. This arrhythmia resolved after administration of intravenous glycopyrrolate.

In none of the procedures did hypotension or hypertension occur. No airway difficulties during emergence or respiratory distress during the postoperative period were observed in any of the survived procedures.

A simple association was noted between the duration of the procedure and the presence of papilloma in the supraglottis, the presence of papilloma in the glottis, and the severity of supraglottic disease. There was a significant correlation only between the duration of the procedure and complications ($p < .001$). Tracheal disease and severity of tracheal disease only approached statistical significance ($p = .051$ and $p = .054$, respectively). A stepwise logistic regression procedure with exclusion of the procedure's duration revealed that no variable had significant predictive value regarding the occurrence of complications.

DISCUSSION

The introduction of suspension laryngeal laser surgery challenged the surgeon and the anesthesiologist with the task of working together in the same area while trying to eliminate the major hazard of combustion. The unique characteristics of the pediatric airway, especially when anatomic structures are altered by papilloma growth, present even more difficult problems.

Endotracheal intubation represents the easiest and safest way to secure the airway. However, standard ETTs may cause severe burn injuries to the airway from accidental combustion from the laser beam. Although various methods of protection have been used to prevent this hazard,[8] none of them are absolutely reliable. In addition to the combustion problem, the ETT limits the visibility of the operating field and the working space available for the surgical procedure. In order to provide full and safe access to the larynx, anesthetic techniques that do not use an ETT have been developed. Apneic anesthesia involves hyperoxygenation of the patient followed by injection of a sedative and paralytic agent to stop respirations.[9] This will allow less than 4 minutes of laryngeal surgery until further ventilation and oxygenation is necessary either by mask or through a reinserted ETT. This process is time-consuming and carries the risk of hypoxemia and hypercapnia. Repeated reinsertion of the ETT may cause laryngeal injury. The introduction of the jet-Venturi technique has provided an effective alternative to endotracheal anesthesia.[10] However, small variations in jet pressure or even minor alterations in the direction of the jet may produce significant variations in gas flow and intrathoracic pressure. Therefore, the possibility of hypoventilation or hyperventilation and the risk of airway barotrauma increase as the size of the airway diminishes.[11] Infants and children in whom the airway is narrowed by RRP may be more prone to these potential complications. Jet ventilation may also cause inflation of the stomach and dehydration of mucosal surfaces.

The major objection to SRA is the potential risk to operating room personnel from the anesthetic gases and laser smoke plume released into the room. Placement of a large suction evacuator near the laryngoscope port may assist in reducing pollution by recovering the outflow contaminated gases. Furthermore, various intravenous agents can be introduced to avoid or reduce the use of inhalation gases that pollute the operating theater atmosphere. Intravenous propofol was used most commonly and was found to be effective in reducing the halothane requirement during anesthesia maintenance.[12,13]

The SRA technique described in this series was proven to be relatively safe and effective in the management of children with RRP who underwent laser laryngeal microsurgery. The absence of a tube in the larynx offers a definite advantage when the laser is to be used. The SRA technique provides an inherently safe means by which the patient with an obstructed airway can breathe spontaneously throughout the procedure. An unobstructed view of the larynx is provided without combustible material in the airway. However, it should be noted that although this technique is considered effective and safe, control of the airway is not absolute and constant vigilance is needed to ensure its patency, especially when it is partially obstructed by the papilloma. Moreover, SRA depends on a carefully balanced anesthetic technique mixture of inhalation gases and intravenous agents to anesthetize the patient without significantly suppressing spontaneous respiration. The depth of anesthesia therefore needs careful monitoring. Obviously, good cooperation between the surgeon and anesthesiologist is essential for the success and safety of the technique.

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BIOMECHANICAL AND HISTOLOGIC OBSERVATIONS OF VOCAL FOLD FIBROUS PROTEINS

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This article discusses the molecular composition of the vocal fold and the relationship of fibrous molecules to the biomechanical and physiological performance of the tissue. The components of the extracellular matrix may be divided into fibrous proteins and interstitial proteins. The fibrous proteins, consisting of collagens and elastins, are the focus of this report. Elastin concentration varies by tissue depth in the vocal folds. Variation of elastin by age is reported, but some controversy exists. The biomechanical terms of stress and strain (and stress-strain curves of human vocal folds) are related to the fibrous proteins of the vocal folds. The fibrous proteins, their role in stress, and their effect on the dynamic range of vocal pitch are presented.

KEY WORDS — age, biomechanics, collagen, elastin, lamina propria, pitch, strain, stress, voice.

The lamina propria (LP) of the vocal fold has received considerable attention from laryngologists over the last 2 decades. Although some interest in this tissue was present before that time, it was Hirano's histologic work, coupled with the introduction of the cover-body theory of phonation, that propelled a basic understanding of the LP to the laryngologist.[1-3] His work was unique, in that his research group not only identified histologic differences in various regions of the LP, but also related those findings to vocal fold physiology with clinical implications.

Hirano[1,2] found that the LP of the vocal fold is made up of 3 layers. The most superficial layer of the LP (SLLP) was characterized histologically by loose tissue with few collagen or elastin fibers. The middle or intermediate layer of the LP (MLLP) was found to have an increased number of elastin fibers, and the deep layer of the LP (DLLP) had an increased number of collagen fibers (Fig 1[4]). The more superficial tissue was termed the cover, and the deeper tissue was called the body.[3]

The notion of a cover and body fit well with studies of vocal fold vibration showing not only medial-to-lateral motion of tissue, but also vertical motion of tissue.[2,5] The physiological concept of the theory is that the more medial and superficial tissue slides, glides, and moves over a more rigid body of tissue. Specifically, a wave of "mucosal upheaval," which starts in the immediate infrafold area and then travels superiorly, had been identified on high-speed cinematography.[6-9] This is referred to as the mucosal wave. It was recognized that for the superficial tissue, freedom of movement in all directions was critical. The cover-body theory of phonation met that criterion, and the studies of the LP provided histologic evidence that the superficial tissues allowed freedom of movement, while the deeper tissues were more tightly integrated or bound.

The cover of the vocal fold included the epithelium and the SLLP, while the DLLP and vocalis muscle were assigned to the body. The MLLP was appropriately characterized as a transitional layer. Since the amount of tissue in vibration depends on inten-

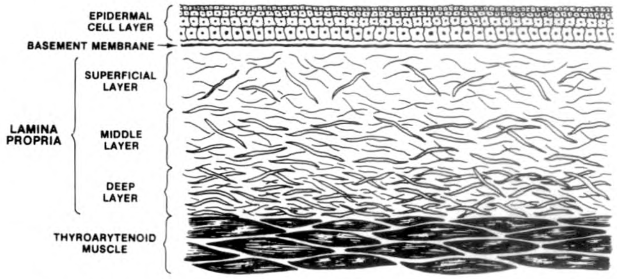

Fig 1. Tissue of vocal fold and its division into layers based on anatomic, functional, or biomechanical differences. (Reprinted with permission.[4])

From the National Center for Voice and Speech (Gray, Titze); the Department of Surgery, Division of Otolaryngology–Head and Neck Surgery, University of Utah School of Medicine, Salt Lake City, Utah (Gray, Hammond); and the Department of Speech Pathology and Audiology, University of Iowa, Iowa City, Iowa (Titze, Alipour). This work was supported by grant P60 DC00976-09 to the National Center for Voice and Speech from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders.

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EXTRACELLULAR MATRIX

Fibrous Proteins	*Interstitial Proteins*	*Other Molecules*
Collagens	Proteoglycans	Lipids
Elastins	Glycoproteins	Carbohydrates

sity and pitch, the cover would appear, at times, to be thicker and would extend to include more of the intermediate layer.[10] Additionally, the normal thickness of the SLLP and MLLP probably varies somewhat according to age, and this would affect what layers of the MLLP and how much of the MLLP is involved in the cover.[11]

Titze[12] gave mathematical proof that self-sustained oscillation of the vocal folds was a direct result of a propagating mucosal wave. The energy transfer mechanism from aerodynamics to tissue movement was shown to be in the form of a negative persistence in the nonlinear differential equation for the notion of the body and cover. The mucosal wave velocity in the cover was responsible for the negative resistance. It needs to be remembered that the cover-body concept is one of the simplest physiological divisions of the anatomic correlates. Another, the mucosa-ligament-body concept, involves 3 functional layers. The vocal ligament was described by Hirano[1] and Hirano and Kakita[3] as the MLLP and the DLLP. The elastin and collagen fibers making up the ligament are arranged in a longitudinal fashion so that they are relatively parallel to the thyroarytenoid muscle. In this arrangement they can respond to longitudinal stress when influenced by the intrinsic muscles of the larynx. Although the fine motor control of phonation and the registers of the voice (falsetto, chest) are determined by the intrinsic laryngeal musculature, the ligament helps to balance the tensions in adjacent layers, helps to set the geometric shape of the tissue to be oscillated, and is a major bearer of stress in more intense and high-pitched phonation.[10]

EXTRACELLULAR MATRIX

Tissues can be divided into cellular and extracellular ones. This division is particularly useful for the vocal folds, since the vast majority of the cover tissue is extracellular, but the majority of the body tissue is cellular (muscle). The extracellular tissue is actually a matrix of proteins, carbohydrates, and lipids (see Table). Although the lipids and carbohydrates are undoubtedly important, they are very difficult to study, since we do not have good, specific, histologic ways of identifying lipids or carbohydrates. The proteins are much easier to identify, and studies of the extracellular matrix (ECM) have divided the proteins into fibrous and interstitial proteins.

The fibrous proteins are the collagens and elastins.[1] The interstitial proteins are the proteins located in the spaces between the fibrous proteins. They are proteoglycans and glycoproteins.[13] Both the interstitial and fibrous proteins have properties that are important to human vocal fold physiology. The fibrous proteins are important for shape and form and are designed to handle stress (a precise biomechanical term discussed below). The interstitial proteins appear to control tissue viscosity, water content, tissue size, and the size and density of collagen fibers.

ELASTIN

Various types of collagens and elastins are found in the LP of the human vocal fold. Elastin material occurs in 3 types, dependent on the ratio of amorphous to fibrillar elastin. The 3 types are elaunin, oxytalan, and elastin fibers.[11] Oxytalan is composed of microfibrils 10 to 12 nm in diameter. Elaunin has these microfibrils and a small amorphous component. Elastin fibers have a larger amorphous component as a core, with the microfibrils surrounding the core. The elastin fibers are sometimes referred to as mature elastin, since of the 3 types, the elastin fibers are the most elastic (biomechanically referring to the ability to be stretched roughly 2 times their length and then return to the normal length).[14] Elaunin and oxytalan are less stretchable and are found in tissue in which stress is higher, such as the superficial layer of tendons and in cartilage.[15] These 2 types are also common in fetal and perinatal tissue, and consequently were initially thought to be immature forms of elastin fibers. That concept is no longer accepted, as elaunin and oxytalan have been found in many tissues in mature humans.[14-16]

Differentiating elastin types is significant to laryngology, in that there is a significant amount of elastin proteins throughout the LP, some of it not in elastin fiber form, but in elaunin and oxytalan form. This is especially true in the SLLP. It is important to note that of the 3 types of elastin material, only the elastin fiber form is stained by most of the histology stains for elastin.[17] Elaunin and oxytalan forms of elastin are not stained well; consequently, these are not often visualized and at the present state of technology can only be fully identified with electron microscopy.

The amount of elastin material in all layers of the LP can be easily appreciated by the following simple experiment. Elastin material (all forms) does autofluoresce when stained with hematoxylin and eosin, and when the LP is made fluorescent, all layers of the LP show much elastin.

Figure 2 shows such a vocal fold LP being made fluorescent. The yellow color is all elastin material, and the immediate SLLP is brightly shining, show-

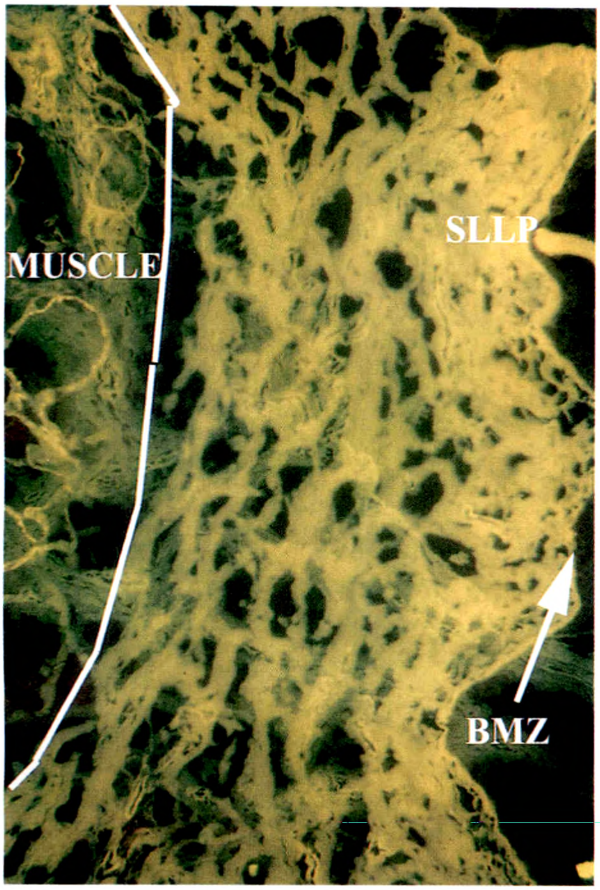

Fig 2. Vocal fold tissue stained with hematoxylin and eosin and then made fluorescent. Elastin molecule becomes fluorescent. Demonstrates tissue between basement membrane zone (BMZ) and vocalis muscle. Specimen was fresh, frozen tissue; hence, there are gaps or dark spaces in between fluorescent tissue. Dark spaces are therefore artifactual and due to technique. This Figure, which indicates that elastin protein is present in considerable abundance throughout all layers of lamina propria, is in contrast to Fig 3, showing that elastin fiber component is more predominant in middle and deep layers. SLLP — superficial layer of lamina propria.

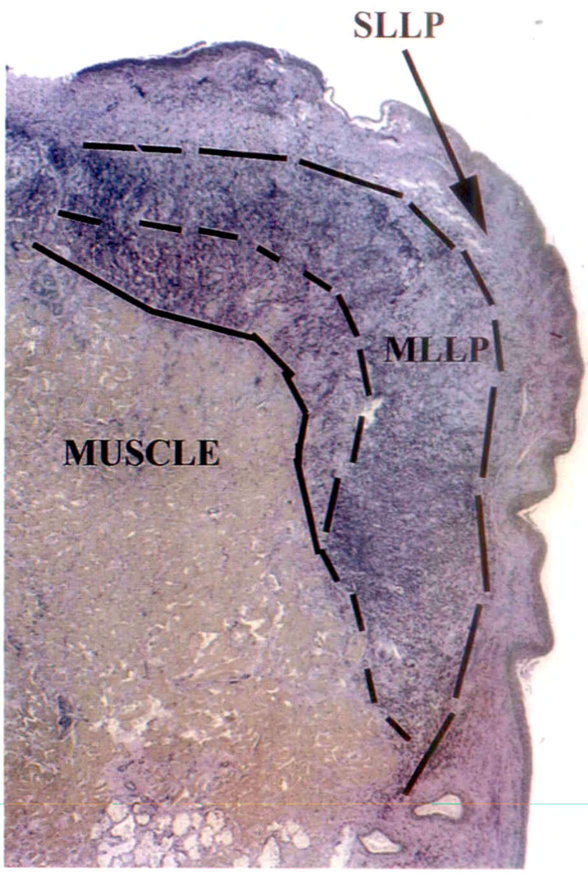

Fig 3. Vocal fold material stained for elastin fibers (elastin Verhoeff's stain). There is preponderance of elastin fibers in intermediate and deep layers. From Figs 2 and 3, one can see why entire lamina propria has elastic qualities, whereas vocal ligament portion has biomechanical properties for stress and strain, as described in text. MLLP — middle layer of lamina propria.

ing abundant elastin to be present in the SLLP. This Figure illustrates that the entire LP contains much elastin. However, when Verhoeff's elastin stain is used to identify only the elastin fibers, few elastin fibers are found in the SLLP; hence, the elastin in the SLLP is not in elastin fiber form. It was the elastin stain that was used to originally identify the 3 layers of the LP. Figure 3 shows the LP stained for elastin fibers.

ELASTIN FIBER DISTRIBUTION IN LAMINA PROPRIA

Verhoeff's elastin staining and an image analysis system have been used to study the concentration of elastin fibers in the LP in infant, middle-aged, and geriatric specimens[18] (Fig 4[11]). The main objective of that study was to identify the concentration of elastin fibers in the various layers of the LP with respect to age and gender.[11]

As can be seen from Fig 4, in normal, middle-aged adults, the slope of elastin fiber concentration starts increasing at about 25% depth into the LP and be-

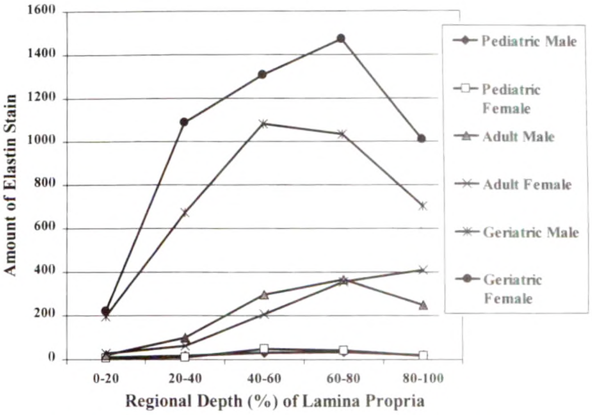

Fig 4. Graph showing increasing elastin fiber concentration by age. Note that even in superficial layer, elastin content is good deal higher in geriatric folds than in younger adults.

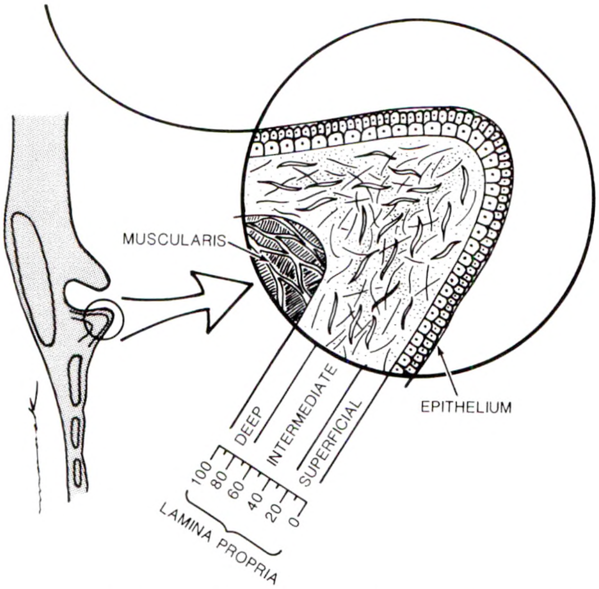

Fig 5. Schematic of estimated width of lamina propria layers based upon elastin fiber concentration. (Reprinted with permission.[11])

gins to level off at about 45% depth. It continues to stay high until the last 15% to 20%. From these data, it appears that the SLLP consists of about 25% to 35% of the initial depth of the LP, with the MLLP making up the next 45% to 55% (Fig 5). Although our tendency was to call the last 20% the DLLP, that percentage may not be entirely accurate, since the study did not assess collagen fiber density. It does demonstrate the magnitude of the differences in concentration of elastin fibers by depth and by age between the SLLP and the vocal ligament. The elastin concentration was much higher in the geriatric samples and lowest in the infants. It was noted that the increase in the elastin fiber concentration includes the SLLP as we get older, and the thickness of the SLLP, measured by this histologic method, becomes considerably thinner in the geriatric patient. Three representative photographs are shown to illustrate these two points: the thinning of the SLLP and the increasing elastin fiber concentration. These specimens received a Verhoeff elastin stain that stains elastin fibers black. The elastin fibers appear as tiny black dots in the LP. Figure 6A is from an infant larynx; note that minimal elastin is present. Figure 6B is from a middle-aged male. Note that the MLLP shows moderate staining of black elastin fibers. Figure 6C is from a geriatric male larynx, and the abundant black staining is apparent almost right up to the epithelium. No gender difference in the amount of elastin fibers across the LP was identified. This finding may be important in guiding us toward issues of replacement therapy of the LP. Perhaps a mixture of proteins such as collagen and elastin would be the best biomechanical LP substitute.

If one believes that limiting surgery to the SLLP avoids scarring, then this study would indicate that the patients at risk for scarring are in the geriatric population. Clinically, this concept may not bear out, as it seems from clinical experience that geriatric patients are not more predisposed to iatrogenic laryngeal injury. It may be that it is not the collagens and elastins of the ECM that determine the limits of surgical safety, but rather, the presence or amount of proteoglycans (hyaluronic acid and decorin) in the SLLP that leads to the ability to operate on the SLLP without inducing scar.[19] If the concentration of certain proteoglycans such as decorin or hyaluronic acid in the SLLP is low, then even minor surgical intervention could potentially lead to scar formation in the SLLP, despite great surgical skill.

COLLAGEN

Collagen types I, II, and III have all been identified in the LP.[4] Collagen types IV and VII are located in the basement membrane zone.[20-23] Collagen studies are somewhat more difficult to do, because within 24 hours of death, collagens lose their antigenicity, making collagen-specific studies unreliable even when done within a few hours postmortem. We do not have quantitative studies of how the collagens are distributed across the LP. The biological function of collagens is fairly singular: to provide strength. Collagens do not stretch well. They do hold things together well. Their general spatial orientation is similar to that of the elastin fibers: directed longitudinally, from anterior to posterior. This orientation allows them to bear the stress of intrinsic laryngeal muscles.

FIBROUS PROTEINS AND SENESCENCE

The finding of increased elastin in the LP with aging is consistent with other studies of connective tissue. As we age, we tend to accumulate both collagen and elastin fibers in our ECM, because the body breaks down and turns over less of these fibrous proteins. Kohn[24] repeatedly pointed out that age-related changes in most organ systems are expressed more in the ECM than in the actual cells.[23] This is demonstrated by tendons' becoming more resistant to heat denaturation and less soluble and digestible by collagenase as we age.[25] The elastic modulus of the lung becomes stiffer and decreases linearly with age.[26] The myocardium becomes stiffer, and arteries become less elastic with age.[27,28] These changes are functions of the components of the ECM. More elastin or collagen does not necessarily reflect better elasticity or function of the tissue, since most of the elastin and collagen in aged tissue is less functional, due to cross-linking.[29]

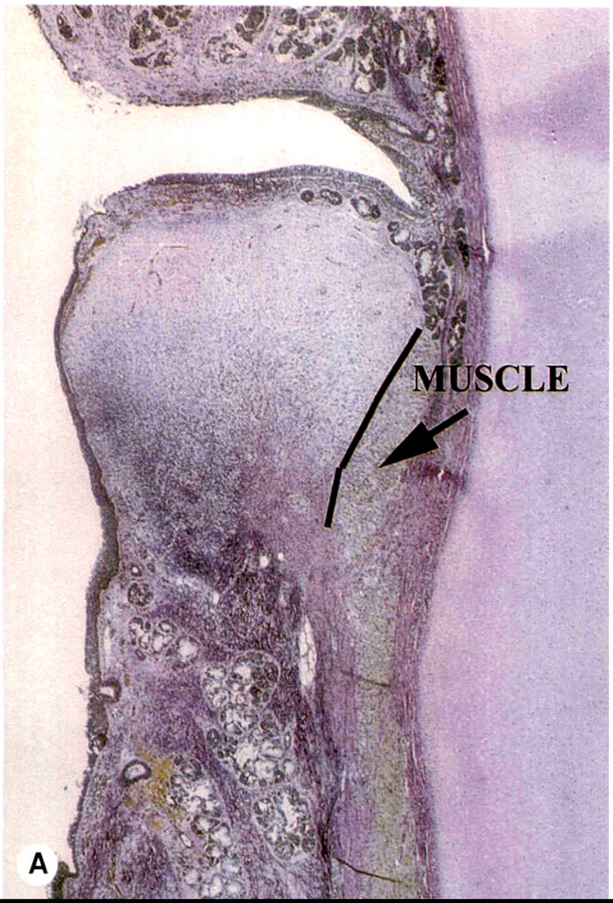

Fig 6. Vocal folds of 3 ages examined with Verhoeff's elastin stain, which stains elastin fibers black. A) Four-month-old boy. B) 43-year-old man. C) 69-year-old man. Note that superficial layer shrinks, and black color becomes more predominant in intermediate layer. Also note that fullness or total width of intermediate layer is not only large at leading edge, but remains substantial slightly below leading edge in immediate infrafold portion.

[redacted]

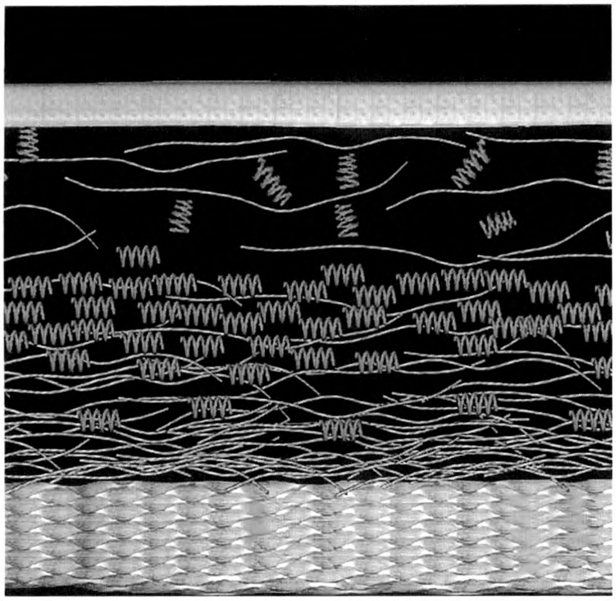

Fig 7. Fibrous proteins of vocal fold demonstrated by computer rendering performed on Animation Master, software package of 3-dimensional modeling and rendering produced by HASH, Inc. Ropelike structures are to represent collagen fibers, and springlike structures are to represent elastin fibers.

LAMINA PROPRIA AND SENESCENCE

These findings are not in complete agreement with other LP studies of elastin. Sato and Hirano[30] showed cross-linking of elastin fibers in geriatric specimens, but reported that elastin fibers seem to become less numerous than in younger adults. Earlier studies by Hirano et al[31] also reflected a decrease in elastin fibers between normal and geriatric vocal folds. We do not know how to explain these differences from the findings of Hammond et al.[11] Similar confusion has been found in other literature, in which elastin content has been reported to increase, decrease, or remain the same through senescence. Although both groups employed elastin stains, the methodology used was quite different. Hammond et al[11] used a quantitative image analysis system and fewer specimens, while the other group used more specimens and qualitative visual assessment. Both methods have some advantages and disadvantages. Perhaps racial differences could be present.

These studies help define how the microarchitecture of the vocal folds changes as we age. Clinically, the knowledge of increased or decreased elastin fibers in the LP is not very useful without knowledge of how that finding may affect vocal performance, which at this time is lacking. The finding of the shift to the left for the steep portion of the elastin curve in Fig 4 for geriatric specimens has several possible implications. Is the SLLP shrinking (atrophy), or is it simply being invaded (conversion) by elastin? Whatever the mechanism, the result is a proportional increase in the total width of the MLLP and a decrease in the width of the SLLP. This decreases the amount of cover in relation to the body of the vocal fold as we age. In the infant, this scenario is reversed, with a very large cover-to-body ratio. This thinning of the SLLP may have relevance to formation of a sulcus or contribute to some cases of bowed vocal folds. Although we do not have a knowledge of what these vocal folds looked like in vivo, occasional specimens have been found that appeared to have a sulcus vergeture, and in these specimens it seems as though the SLLP is lacking, with the MLLP and DLLP being relatively normal. The MLLP's superficial border seems to be nearly the basement membrane zone in severe sulcus cases. This finding could explain the profound effect a sulcus may have on the voice. Losing the SLLP may be tantamount to loss of most of the functional tissue cover. Instead, the epithelium really is attached directly to a transitional layer and the body of the vocal fold. Anecdotally, we have not seen any cases in which atrophy of the MLLP seems to occur. From our observations, it also appears that some conversion of SLLP to MLLP is occurring. This concept would be supported by general theories of aging in which it has been reported that as we age, the elastin content of the ECM in some tissues slowly increases.[25,32] It is likely that aging may produce both atrophy of the SLLP and some conversion (infiltration) of the SLLP by MLLP proteins. We need to reemphasize that we do not know how these folds appeared and performed when in vivo, and these are strictly histologic observations.

The ECM is conceptually built as shown in Fig 7. Thus far, we have discussed only the fibrous proteins of the ECM (collagens and elastins). We have reviewed the distribution of elastin fibers through the LP as it relates to age. Note that there are more collagen fibers deeper in the fold. Note also that the overall arrangement of the fibrous proteins is parallel to the edge, although individual fiber molecules may not be so directed. Recall that elastin material is present throughout all layers, although the elastin *fiber* form is most concentrated in the MLLP.

BIOMECHANICS OF FIBROUS PROTEINS

Biomechanically, stress-strain curves are frequently used to describe properties of fibrous materials.[10] Stress can be defined as the amount of force per unit area applied to a substance to induce a physical, morphological change. Strain is defined as corresponding deformation (the change in length minus the original length). Studies on the vocal ligament have measured how much stress leads to a certain level of strain.[33,34] Figure 8 (stress-strain) illustrates this re-

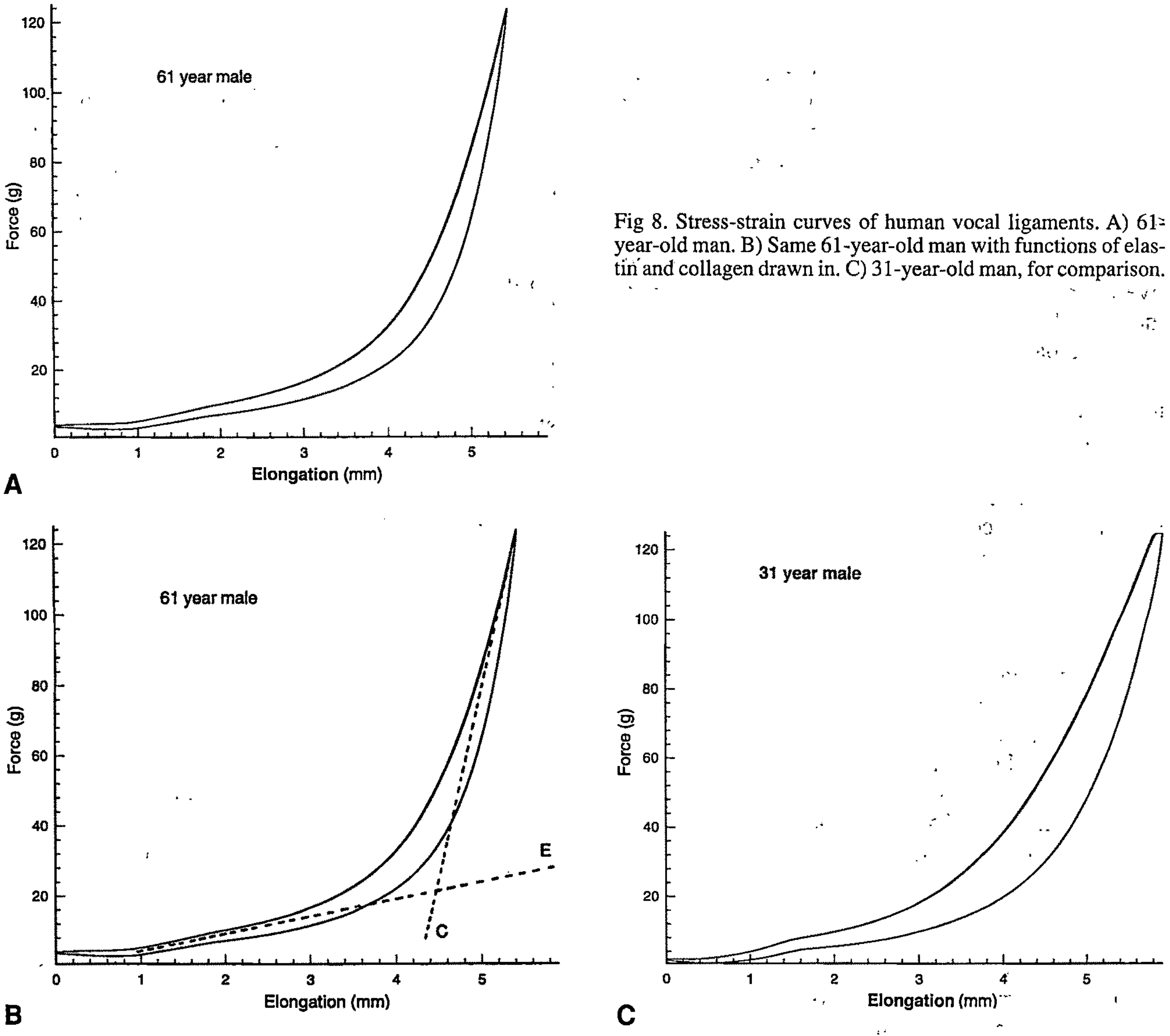

Fig 8. Stress-strain curves of human vocal ligaments. A) 61-year-old man. B) Same 61-year-old man with functions of elastin and collagen drawn in. C) 31-year-old man, for comparison.

lationship. These stress-strain curves were obtained according to standard methodology.[34]

Figure 8A is a stress-strain curve of a 61-year-old man. Figure 8B is the same curve, but with lines drawn in to show linear approximations to the collagen and elastin fiber responses. The last stress-strain curve is from a younger, 31-year-old man for comparison (Fig 8C). Note that the two curves are not exactly similar. The stress-strain curve of the 61-year-old man shows a steeper slope in the collagen portion (increased stiffness) and less area between the lines, indicating less tissue creep (increased viscosity) than in the 31-year-old man.

When stress is applied longitudinally to the fibers of the vocal ligament, the ligament stretches to a longer length. Note from the stress-strain curves that the vocal ligament stretches easily at a low level of stress, but upon reaching a longer length, the stress required for further lengthening increases remarkably. This type of nonlinear response is attributed to the mix of collagen and elastin fibers.[32] The resistance to stretch due to elastin alone, making the first part of the stress-strain curve fairly linear (springlike), characterizes the initial part of the stress-strain curve in the human vocal ligament. The early part of the stress-strain curve mirrors elastin fiber performance. After a certain amount of strain has occurred (about 30%), the stress-strain curve takes a noticeable upward slope. In stress-strain relationships of ligaments and tendons, the point at which the upward slope occurs is often referred to as the "break-point," because it represents the place of ligament strain in which collagen fibers are being recruited to resist further strain.[35] Once the break-point area is reached, further stress does not result in much more length change within the vocal ligament. The late part of the stress-strain curve is similar to collagen fiber performance. Note that in the 61-year-old man, the part of the curve corresponding to collagen fiber performance is steeper than in the 31-year-old man. This likely indicates increased stiffness in the collagen fibers of the older man, a common finding as we age. Also note that the area within the 31-year-old's curve is greater than that of the 61-year-old. The area within the curve is an indirect indication of viscosity. The 31-year-old man's vocal ligament likely has lower viscosity and more tissue creep than that of the 61-year-old man.

The steeper part of the stress-strain curve, reflective of the collagen fiber composition, shows how the vocal ligament resists strain but facilitates pitch rise. The particular composition of the vocal ligament varies individually and with age. With respect to vocal performance, it is interesting to reflect on how the response of elastin is meshed with the response of collagen to make the stress-strain curve of the vocal ligament. The interaction between elastin fiber performance and collagen fiber performance may be influenced by proteoglycans.

DYNAMIC RANGE PROPERTIES OF FIBROUS PROTEINS

For speaking pitches, the more functional part of the stress-strain curve, the part of the curve that most of us vocally use daily, is dominated by elastin fiber performance. For singing at high pitches, however, the contribution of collagen to the stress-strain curve may be significant. To understand this, we appeal to the formula for the frequency of a vibrating string:

$$F = \frac{1}{2L}\sqrt{\frac{\sigma}{P}}$$

where σ is stress, P is tissue density, and L is the length of the string.[10] The authors recognize that the vocal folds cannot be adequately described as vibrating strings, but the mathematical relationships described by this equation do have some merit in helping us understand biomechanical relationships in phonation. As can be seen from the equation, length has an inverse relationship to frequency. That is, although the vocal folds become longer as our pitch goes up, the longer vocal folds should vibrate at a lower frequency. To raise the frequency, we have to overcome the increase in vocal fold length by increased stress. Tissue density is nearly a constant (1.0 to 1.05 g/cm^3). By increasing tissue stress by a factor of 4, we double the frequency. (Note the square root relation.) But this rise is diminished by the length increase, giving us less than a doubling of frequency for a fourfold increase in stress. Now it is apparent why the stress-strain curve is so relevant to vocal production. It is critical that we easily generate high levels of tissue stress without much increase in length. This ability to easily generate high levels of tissue stress is a product of the collagenous part of the vocal ligament and is a main mechanism of providing humans with a large dynamic pitch range.

SUMMARY

In summary, the proteins of the ECM of the vocal folds are classified into fibrous and interstitial proteins. The collagens and elastins constitute the fibrous proteins and influence the stress-strain properties of the vocal folds. Collagens are proteins that bear stress and contribute strength to the tissue, whereas elastins are proteins that allow the tissue to deform and return to its original shape. Collagens and elastins together likely convey certain biomechanical properties to the vocal ligament that may change with age. Tissue stress, the ability to carefully control it and precisely generate it, is important for dynamic pitch range in humans.

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ENCOUNTERS WITH CHALLENGING BRONCHIAL FOREIGN BODIES: IMPROMPTU ADAPTATION OF TECHNIQUE

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We present a retrospective review of 2 cases of unusual foreign bodies and discuss methods needed for successful removal of the objects. The review stresses the need for preparation in managing difficult and unexpected situations. Specific instrumentation as well as surgical resourcefulness provided for successful removal of a carrot fragment in one instance and a plastic bead in the other. The need to individualize tracheobronchial foreign body extraction is stressed, as is the importance of keeping some of the "old-time" instruments in one's instrument cabinet.

KEY WORDS — aspiration, bronchus, foreign body extraction.

> "Aber nein. Das geht nicht. You can't do dat. Imposseebla."
>
> A rude interruption of my writing by thunderous sounds suggestive of the pounding to pieces of the old gristmill had brought me face to face with the old millwright. "He got a nail-spike in hees gitzard"—pointing to the corn-and-cob crusher.
>
> "He got to come down."
>
> "Can you not reach the spike from above?"
>
> "Nein. Ich bin too short on one end. He gotta come down."
>
> "There is no need of taking down the whole mill to get a spike out of the crusher."
>
> "Gott verdammier foolishment. Imposseebla," said the millwright as he saw the lowering of a flashlight down into the "gizzard," of the mill, followed by a length of gas pipe with an improvised wire snare loop through the pipe. The loop encircled the spike and when tightened held it securely for withdrawal.
>
> "Ach so-o-o! Sehr gut. I learn somet'ing a'ready dis morning' yet."[1(p149)]

The above quote from Chevalier Jackson's autobiography is an illustration of how resourceful he was. Many of us know that when he needed a particular instrument, he would simply call Mr Pilling, and the instrument company would immediately create whatever instrument he had suggested. The title of this paper suggests that the endoscopist, even today, must be resourceful.

Infants and toddlers younger than 5 years of age account for approximately 84% of foreign body aspirations. The fact that 73% of the 84% are in children under age 3 reflects the tendency of younger children to explore their surroundings and satisfy their oral needs.[2] Considerable morbidity still exists when the principles of management so meticulously developed by Dr Jackson are not followed. Every endoscopist should have practiced preoperatively using a tracheal board and, if possible, anesthetized animals. It is important to try to simulate the foreign object when practicing object removal. This enables the endoscopist to select the appropriate instrument for each particular object. In spite of these important preparatory steps, unusual presentations may occur that challenge the endoscopist to be innovative. This paper will review 2 such instances in which "standard practice" was insufficient for extraction of the foreign object.

TECHNIQUE

Most cases of foreign object removal can and should be performed under general anesthesia. We prefer to have the patient breathing spontaneously. The technique can be enhanced by anesthetizing the vocal cords with topical Xylocaine, 4 to 5 mg/kg. The instruments are selected on the basis of the patient's age and the expected foreign object. If a peanut is suspected or the object has been present for more than 24 hours, a loading dose of corticosteroids is given in an effort to decrease the inflammatory response. Most tracheobronchial foreign bodies can be removed with the fenestrated peanut forceps or the forward-grasping optical forceps.

CASE 1

A 2-year-old child had a sudden coughing paroxysm while playing with some plastic beads and other plastic toys. He was not cyanotic, and the parents thought he had probably swallowed a plastic object. After experiencing intermittent fever for 6 days, the

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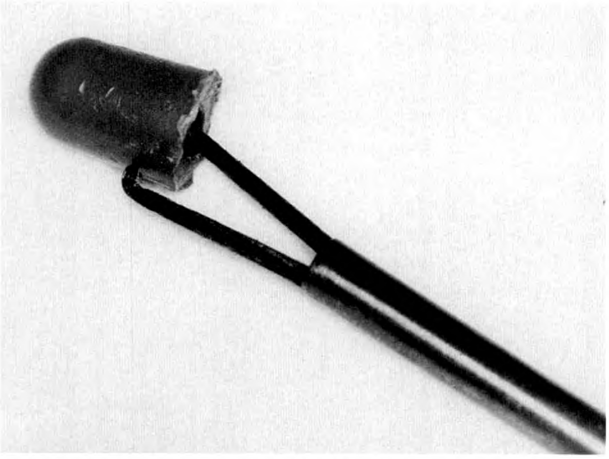

Fig 1. Jackson sharp-pointed right-angle rotation forceps holding object from case 1 (Pilling Company, Fort Washington, Pa).

boy began coughing and wheezing. A chest radiograph revealed right middle lobe and right lower lobe atelectasis and a slight deviation of the trachea to the right side. The white blood cell count was 30,000. Since the object was believed to be plastic and minimal inflammatory response was anticipated, the usual preparation was made for removal. A 4-mm × 30-cm Storz pediatric bronchoscope was introduced. The trachea and left main bronchus were normal, but just distal to the right upper lobe orifice, the bronchus was occluded by granulation tissue. With a metal bronchial suction tube, the granulation tissue was fragmented, and the bronchoscope was then cored into the mass until a red object was barely visualized. Bleeding associated with this granulation tissue was controlled with instilled oxymetazoline hydrochloride. The object appeared to be a plastic bead with a hole in the center. Because of the marked inflammatory response, the object was tightly wedged in the bronchus. Several attempts were made to grasp it with the fenestrated forceps, but the object could not be displaced. A colleague observing the procedure then searched the bronchoscopic instrument cabinet and gave the author (W.S.G.) one of Dr Jackson's original Pilling-designed Jackson sharp-pointed right-angle rotation forceps (Pilling Company, Fort Washington, Pa; Fig 1). This instrument grasped the center hole of the object with 1 tooth while its other tooth grasped the periphery. A significant effort was required to dislodge the object and eventually deliver it. A large amount of purulent material was present distal to the obstruction, but the remainder of the bronchus showed only granulation tissue. The child was observed for 2 days in the hospital and showed no further problems.

CASE 2

An 18-month-old child presented with a 1-week history of having choked on some carrot fragments. He was coughing and had developed a temperature elevation. He was referred for evaluation of suspected foreign body aspiration. Pertinent findings on chest examination included wheezing and decreased breath sounds on the right side. Chest fluoroscopy showed sluggish motion of the right hemidiaphragm. Bronchoscopy was performed with a 3.5-mm × 20-cm infant bronchoscope. The trachea and both main bronchi were patent. The lateral segment of the right lower lobe revealed a tiny fragment of carrot lodged distally. A forward-biting forceps could not be introduced because of edema and the distal location of the carrot. One milliliter of 0.25% phenylephrine hydrochloride was placed at the site in an attempt to reduce the swelling. A small bronchial suction tube with a flared tip was advanced until the object was palpated (Fig 2). Suction was then applied and the carrot fragment was engaged in the flared tip and safely removed. On further examination, no other foreign body was found. The child did well postoperatively.

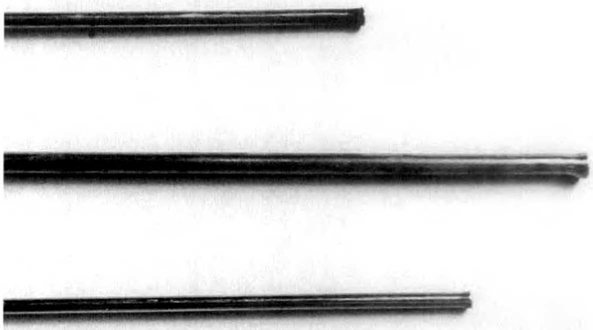

Fig 2. Examples of 3 sizes of standard bronchial suction tube with flared tip as used in case 2. (Adaptation of Dr F. Davison, deceased, formerly of Geisinger Medical Center.)

DISCUSSION

These 2 cases of bronchial foreign body removal demonstrate the occasional need for special instrumentation as well as surgical resourcefulness. In the first case, the use of an instrument that is not even designed for the removal of bronchial foreign bodies was necessary. In fact, it proved to be the only instrument strong enough to extract the object. In the second case, a standard bronchial suction tube had the tip flared by our machine shop (Dr F. Davison's innovation), as depicted in Fig 2, and thus allowed a greater surface area for adherence of the object. In order to gain access to the distal edematous area, we instilled 0.25% phenylephrine. This simple maneuver has proven beneficial in many other cases in which edema or bleeding was encountered.

SUMMARY

Removal of tracheobronchial foreign bodies continues to be challenging for the otolaryngologist. When basic principles of foreign body management are followed, the majority of cases have a successful outcome. Occasionally, it is necessary for the surgeon to be innovative and thus be familiar with all of the instrumentation available to him or her. This paper stresses the importance of retaining the "old-time" instruments in the bronchoscopic cabinet.

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FIFTH INTERNATIONAL CONFERENCE ON HEAD AND NECK CANCER

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MUCOSAL LEISHMANIASIS: QUANTITATIVE NASAL CYTOLOGY AS A MARKER OF DISEASE ACTIVITY AND INDICATOR OF HEALING

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Mucosal leishmaniasis (ML) follow-up is based on subjective parameters. Using simplified quantitative cytology of nasal lavages (QNCs), we studied 20 ML patients, 10 patients with cutaneous leishmaniasis (CL), and 10 healthy subjects. Patients with ML were treated with antimony and followed up with otolaryngological examination plus QNCs for 6 months. At the first evaluation, the median total number of cells in ML patients (1,540,000) was greater than that in CL patients (215,000) or that in healthy subjects (250,000). Neutrophils were predominant in ML patients, in contrast to both sets of controls, in whom epithelial cells were more frequent. During treatment, we found a significant reduction in total nasal cell counts in ML patients who were cured, and encountered a switch in predominant cell type. The cytology of 2 patients who did not respond to antimony remained the same. It is therefore possible to detect nasal inflammation in ML patients through QNCs, which may indicate extension of mucosal involvement, providing an objective parameter to monitor therapy.

KEY WORDS — leishmaniasis, mucosal leishmaniasis, nasal cytology.

INTRODUCTION

It is estimated that worldwide, 400 million individuals are at risk for *Leishmania* infection, with an annual incidence of 600,000 new cases and a prevalence of 12 million ill subjects.[1] Some species of *Leishmania* are capable of causing cutaneous leishmaniasis (CL), while others provoke visceral disease. In areas of transmission of *Leishmania brasiliensis*, one of the agents of CL in the New World, a small proportion of subjects may develop mucosal leishmaniasis (ML). It involves predominantly the nasal mucosa and the nasal septum. In the endemic area of Corte de Pedra, Bahia, Brazil, epidemiological, clinical, and immunologic studies have been carried out for 15 years.[2] In a small health post that serves the village, we see up to 600 cases of CL and 50 cases of ML a year. Mucosal involvement in leishmaniasis is associated with nasal discharge and obstruction. Perforation of the septum is the most common complication of ML. In advanced cases, destruction of the nose, palate, and tissues of the larynx may be observed. Laryngeal damage results in a hoarse, weak voice and may occasionally lead to airway obstruction and death.[3]

Routine diagnosis of ML in endemic areas comprises 3 criteria: 1) a history of CL; 2) characteristic mucosal lesions; and 3) a positive intradermal skin test (delayed hypersensitivity reaction) to *Leishmania* antigens. The combination of these 3 criteria in this type of population has a high positive predictive value.[4] However, the detection of IgG antibody against *Leishmania* may replace the skin test as a marker of infection. Histopathologic examination of biopsy fragments and tissue culture are not sensitive enough for clinical diagnosis, since *Leishmania* may be detected in more than 50% of specimens only when special techniques of tissue culture are employed.[3] Treatment with parenteral pentavalent antimony is prolonged, and recurrence of mucosal lesions is frequent.[5,6] Follow-up of cure is based on clinical judgment, and there is no objective laboratory marker of remission available.

Quantitative nasal cytology techniques have been useful tools for investigation of nasal inflammation under various conditions since the work of Bascom et al[7] in 1988, in which a nasal cellular influx was observed following antigen provocation. We have simplified their procedure enough to make it feasible in clinical settings.[8]

OBJECTIVES

1. To evaluate nasal mucosal inflammation in ML patients through simplified quantitative cytology of nasal lavages (QNCs).
2. To observe nasal cytology changes during treatment and healing of mucosal lesions.

From the Immunology Service (Guerreiro, Barral, Carvalho), the Division of Pulmonary Medicine (Cruz), the Division of Otolaryngology (Lessa), and the Department of Medicine (Rocha), University of Bahia School of Medicine, Salvador, Brazil. This project was supported by Agência Financeira de Projetos (FINEP), Programa de Apoio aos Núcleos de Excelência (PRONEX), Brazil; and National Institutes of Health (USA) grant AI 30639.

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DESIGN, MATERIAL, AND METHODS

Design, Recruitment, and Ethics. The first part of this study was cross-sectional, analyzing QNCs results of 20 subjects with ML at 1 time point before treatment, and comparing them to results of control groups of 10 individuals with CL and 10 healthy volunteers. The second component was a 6-month longitudinal observation of the 20 patients with ML being followed up at monthly intervals. All subjects signed informed consents, and the study protocol was approved by the University Hospital Ethics Committee. Volunteers were reimbursed for transportation and meal expenses when traveling to the University Hospital. Patients were selected from subjects attending the village health post of Corte de Pedra, Bahia, during 1992 and 1993, when a total of 44 patients with ML was registered. Selection of patients (above 10 years of age) for the study was related to our monthly visits to the field, in which an otolaryngological examination was performed in individuals with otolaryngological complaints. The healthy subjects (n = 10) were young adults (median age 22 years; 6 men and 4 women) recruited among medical students and laboratory personnel.

Inclusion Criteria. The following 3 criteria were present for ML (20 patients): 1) CL at present or in the past; 2) lesions of the upper aerodigestive tract compatible with active leishmaniasis; and 3) a skin reaction greater than 5 mm to *Leishmania* antigens or a positive serologic test.

The following 3 criteria were present for CL (10 patients): 1) active CL at present; 2) absence of lesions of the upper aerodigestive tract compatible with active leishmaniasis; and 3) a skin reaction greater than 5 mm to *Leishmania* antigens or a positive serologic test. Identification of the parasite was not attempted, since low sensitivities of both culture and biopsy have been reported.

Exclusion Criteria. Exclusion criteria were an upper respiratory tract infection within the last 30 days, pregnancy, or any physical or mental disability that would not allow full cooperation with the protocol procedures.

First-Visit Evaluation. A first-visit evaluation was performed at the University Hospital in Salvador, 270 km from Corte de Pedra, where the patients were recruited, and included a standard questionnaire, a physical examination, an otolaryngological examination, serology, a skin test with *Leishmania* antigens, and QNCs for patients with ML or CL. In addition, 11 patients with ML underwent nasal biopsies and culture of the aspirates of the mucosal lesion for *Leishmania.* A direct Gram's smear and culture of nasal swabs and nasal lavages were also obtained in 13 patients with ML and 7 patients with CL.

An intradermal skin test with soluble *Leishmania* antigens was performed by the injection of 0.1 mL of antigen (25 μg) on the anterior part of the forearm, as previously described.[9] Then, an indirect immunofluorescence test was done to detect antibodies against *Leishmania.* Since the sensitivity of the immunologic tests for diagnosis of CL is quite variable, depending on the duration of the disease (in patients with cutaneous lesions of less than 3 months' duration, the sensitivity of serology may be as low as 50%, whereas it may be higher than 80% in older lesions), and since there is cross-reactivity between *Leishmania* and *Trypanosoma* (an infection not prevalent in the area of Corte de Pedra) antigens in serology, the majority of our cases were defined by a positive skin test.

A Gram's smear of a nasal swab was followed by a culture of a nasal swab and of nasal lavage fluids for aerobic bacteria in MacConkey and blood-agar media (Difco). *Staphylococcus* species were identified by Staphy-test (Probac, Brazil). Needle aspirates of nasal lesions were performed with 20-mL syringes containing LIT (liver infusion tryptase) medium plus penicillin and streptomycin, and subsequently put in NNN (Nicolle, McNeal, Novy) medium plus 10% rabbit blood, as described previously.[10]

After paraffin fixation, standard hematoxylin-eosin staining was performed.

Treatment. Patients with ML or CL were treated with 1 series (20 days) of pentavalent antimony (Glucantime) injections, 20 mg/kg weight. Those who failed to respond to this initial therapy received up to 4 series of antimony.

Monthly Follow-up Field Visits. Every month, all ML patients included in the study were reviewed in the field, at the health post of Corte de Pedra. These visits included a nasal symptoms questionnaire, an otolaryngological examination, a nasal swab for regular cytologic examination, and a nasal lavage for total cell count as performed in QNCs.

Nasal Cytology. At the first evaluation, nasal cytology was performed by 2 different techniques in all subjects: 1) regular smears of nasal swabs, bilaterally, and 2) QNCs. This latter technique involves nasal lavages with a total volume of 15 mL of saline divided into each nostril in a 2-step procedure (5 mL followed by 10 mL). The recovered nasal fluids from the 5-mL lavage were processed for microbiological studies, and the 10-mL lavage was vigorously shaken to break mucus and then centrifuged at 2,500 rpm for 15 minutes at 4°C. The supernatant was decanted, and the total number of cells was counted from the

TABLE 1. CHARACTERISTICS OF 20 PATIENTS WITH MUCOSAL LEISHMANIASIS

Age (y)	*Sex*	*Duration of ML (mo)*	*Types of Lesions*	*No. of Lesions*	*Location of Lesions*	*Serology Titer*	*Skin Test (mm)*
One lesion only							
15	M	1	Granulomatous	1	Septum only	1:16	6
24	F	2	Granulomatous	1	Septum only	1:64	24
28	M	2	Granulomatous	1	Septum only*	1:32	20
46	M	2	Granulomatous	1	Septum only	1:128	14
47	M	1	Granulomatous	1	Septum only	1:32	15
60	M	3	Granulomatous	1	Septum only	1:256	15
72	M	2	Ulcerated	1	Septum only	Negative	14
Multiple lesions†							
16	M	3	Granulomatous	2	Turbinate	1:64	20
20	M	2	Granulomatous	5	Turbinate, vestibule	1:16	25
27	M	8	Granulomatous	2	Turbinate	1:16	33
30	M	6	Granulomatous	4	Vestibule	1:64	14
28	F	96	Perforation	2	Turbinate	1:64	28
30	M	6	Perforation	2	Turbinate	1:64	15
31	M	60	Perforation	2	Turbinate	1:128	10
34	M	1	Granulomatous	3	Nasal floor	1:16	22
40	F	36	Perforation	3	Turbinate, floor	1:64	10
44	M	1	Granulomatous	2	Turbinate	1:64	12
44	M	3	Ulcerated	4	Turbinate, vestibule	1:256	6
55	M	3	Granulomatous	3	Palate	1:1,024	20
60	M	144	Perforation	3	Larynx	1:1,024	15

*Active lesions in face and nasal columella.

†Multiple lesions always included nasal septum.

sediment with a hemocytometer (Neubauer's chamber). The sediment was resuspended (diluted) in saline in order to have a maximal concentration of 500,000 cells per milliliter, and 200 µL of this cell suspension was placed in each cytospin funnel. Cytospin slides were air-dried and then stained with Diff-Quick (American Scientific, USA) for cell differentials. This technique, which we call QNCs, is a modification of the procedure described by Bascom et al.[7]

Statistical Analysis. For comparing continuous variables, we chose nonparametric tests, because we cannot ensure a normal distribution of our data. For categorical comparisons, Fisher's exact test with Yates' correction was applied.

RESULTS, OBSERVATIONS, AND ANALYSIS

General Characteristics of Patients Studied. The ML patients presented with a wide range of mucosal involvement, varying from 1 lesion only in the nasal septum (mostly granulomatous with no ulceration) to multiple lesions affecting the septum, turbinate, vestibule, nasal floor, palate, and larynx. The subgroup presenting with multiple lesions had ulcerations or perforation more often, as one can see in Table 1. The median age of the ML patients was 32.5 years. The duration of ML lesions was 3 months (median), and the median skin test reaction to *Leishmania* antigens was 15 mm. In the ML group, 17 of the 20 patients were male. Concomitant active CL was found in 9 of the 20 patients with ML. Among the 10 CL patients with no ML taken as controls, the median age was 20.5 years, the duration of CL lesions was 1.5 months (median), and the median skin reaction to *Leishmania* antigens was 13 mm. In this latter group, 8 of the 10 were male. None of these differences was statistically significant, nor was there any difference between these groups and the healthy controls. An immunofluorescence antibody test performed before treatment was positive in 80% of individuals with ML and 70% of subjects with CL. After treatment, it was repeated in 15 of the patients with ML presenting titers equal to or over 1:32 previously, being reduced in only 8 of them (53%).

Microbiology. Culture of nasal specimens for bacteria was performed in 13 subjects, allowing the growth and identification of *Staphylococcus* in all cases. In 7 of the 13, *Staphylococcus aureus* was identified, whereas the remaining 6 had coagulase-negative *Staphylococcus*. β-Hemolytic streptococci group A was found in 4 subjects in conjunction with *Staphylococcus*. Identification of bacteria by Gram's stain

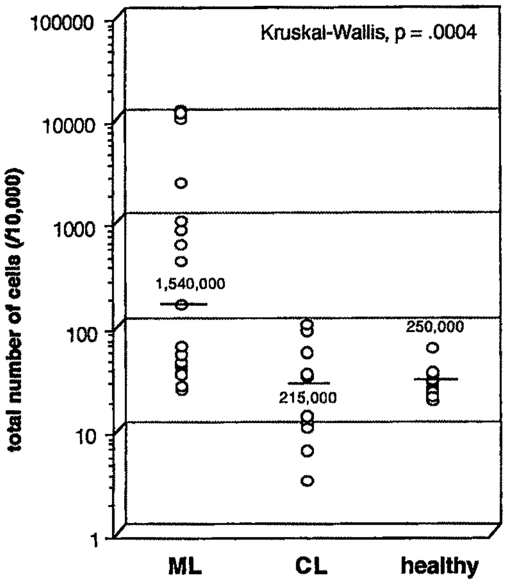

Fig 1. Total number of cells recovered in nasal lavages with 10 mL of isotonic saline in subjects of all 3 groups investigated: active mucosal leishmaniasis (ML), cutaneous leishmaniasis (CL) with no mucosal involvement, and healthy subjects. Horizontal bars and adjacent numbers indicate median values for specific group.

of nasal smears was in agreement with culture results. Gram's stain and culture of nasal specimens of 7 subjects with CL taken as controls demonstrated similar flora.

Histopathology. Observation of the biopsy fragments of 11 patients with ML from whom adequate material was obtained always demonstrated a mononuclear inflammatory infiltrate (100%), and less often, acanthosis (45%) and pseudo-epitheliomatous hyperplasia (27.3%). Frequent eosinophils were seen in only 2 patients. Granulomas with epithelioid cells were seen in 6 cases (54.5%). Only 3 of 11 patients with ML had *Leishmania* in the tissue examined.

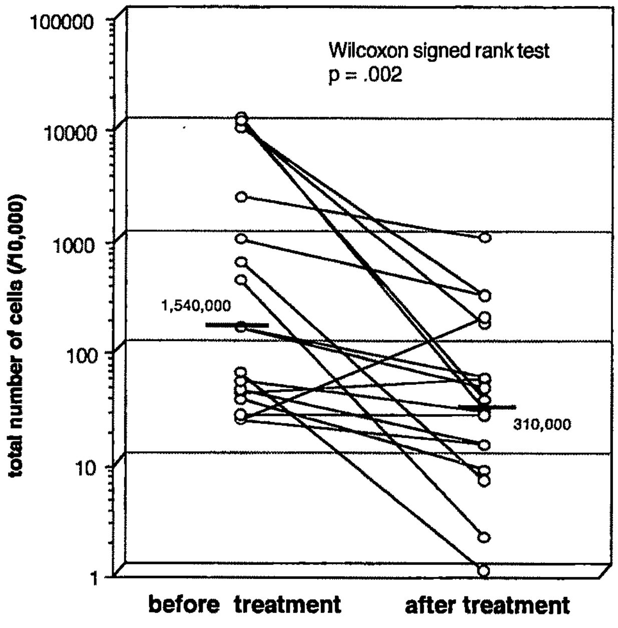

Fig 2. Total number of cells recovered in nasal lavages with 10 mL of isotonic saline in 18 subjects with mucosal leishmaniasis who healed their nasal lesions during therapy with antimony. Horizontal bars and adjacent numbers indicate median values.

TABLE 2. PREDOMINANT CELL TYPE IN NASAL MUCOSA OF PATIENTS WITH LEISHMANIASIS THAT HEALED WITH THERAPY

	Before Treatment	*After Treatment**
PMN neutrophils	12/18 (67%)	2/17 (12%)†
Epithelial cells	6/18 (33%)	15/17 (88%)

*One patient had equal proportion of polymorphonuclear (PMN) neutrophils and epithelial cells after treatment.

†p = .02 (McNemar χ^2 test with Yates' correction).

Total Number of Cells in Nasal Lavages. As depicted in Fig 1, the total cell counts in recovered fluids after nasal lavages were as follows (medians): ML 1,540,000; CL 215,000; and healthy controls 250,000. The total cell count in ML patients was significantly higher ($p < .01$) than that in either control group. A comparison between patients with CL and healthy subjects found no significant difference. After treatment, the median number of cells in nasal lavages of ML patients was reduced to 480,000 — 3.2 times less than before, as shown in Fig 2. This difference was statistically significant ($p = .002$).

Differential Cytology. Epithelial cells were predominant in control subjects, either in cytospin slides of nasal lavages or in smears of nasal swabs. All slides had 100% epithelial cells in patients with CL and healthy subjects. Among the 20 patients with ML, however, at the initial evaluation, 13 had more polymorphonuclear (PMN) neutrophils than epithelial cells in cytospin slides, and 12 had more PMN neutrophils than epithelial cells in any (right or left side) smears. The percentage of PMN neutrophils ranged from 0 to 100, and no eosinophils were found except for 1 ML individual and 1 CL individual. Before treatment, the differential cell counts on cytospin slides obtained from nasal-recovered lavage fluids of ML subjects demonstrated on average 56% PMN neutrophils, 41% epithelial cells, and 3% mononuclear cells. Four of the 7 ML patients with lesions restricted to 1 site had PMN predominance specifically at the site affected. Among the 13 ML patients with multiple lesions, 8 had PMN predominance of any site. After therapy, 2 patients with ML remained with PMN predominance in nasal swabs. Means of 13% PMN neutrophils, 80% epithelial cells, and 7% mononuclear cells were found. There was agreement between cytospin and swab slides in 75% of the patients examined. We found no association between cytology and bacteriologic findings. Table 2 summarizes the results of differential cytology performed before and after therapy in ML patients responding to therapy.

DISCUSSION

Mucosal leishmaniasis usually affects people in rural areas, where medical resources are scarce. Diagnosis is usually based upon endemicity, medical history, physical examination, and skin test. No objective parameter has been used to assess nasal inflammatory changes or monitor response to therapy. Serology is not specific to ML, nor is it sensitive to short-term healing in response to therapy, so that it is not a good test for monitoring treatment. Before treatment, ML patients had higher total cell counts in nasal lavages than CL patients and healthy volunteers. After treatment, the total cell count was significantly reduced in ML patients. We have shown, therefore, that a relatively simple and noninvasive technique, collecting nasal secretions through lavages, may offer a quantitative method useful for evaluating nasal inflammation in active ML. Reduction in total cell count in nasal lavages is associated with the healing of mucosal lesions. Moreover, ML cure was also associated with a reduction in the proportion of PMN neutrophils found before treatment.

Quantitative nasal cytology was widely used in pathophysiology studies by Naclerio's group[7]; however, it was too complicated for clinical practice. We have eliminated many steps of this laborious technique without losing the ability to detect cytologic changes due to nasal provocation.[11] By using QNCs, it was possible to observe marked changes in nasal cytology in response to topical steroids in patients with allergic rhinitis.[12] It is noteworthy that distribution of cell types in nasal lavages may not correspond to biopsy findings in nasal mucosa. Mononuclear inflammatory cells seem to remain in tissue, whereas PMN neutrophils and eosinophils appear to migrate more readily into nasal secretions.[13]

The pathogenesis of ML is unknown. A T-cell response associated with γ-interferon (γ-IFN) production is the main immunologic defense mechanism against *Leishmania*.[14,15] Since patients with ML have evidence of a strong T-cell response to *Leishmania* antigens and their macrophages have the ability to kill *Leishmania*,[2] the hypothesis of a hypersensitivity reaction to explain this presentation of the disease has been considered.[5] The possibility that a locally exacerbated immune reaction is responsible for mucosal lesions in leishmaniasis is attractive. Therefore, specimens collected by nasal lavages can contribute to studies on nasal effects in ML subjects. Herein we documented a predominance of PMN neutrophils in nasal lavages of patients with ML. Since the majority of the patients had nonulcerated lesions in the nose, and bacterial isolates belonged to normal nasal flora in a great number of cases, it is conceivable that the PMN neutrophil predominance in ML is related to *Leishmania* infection. In fact, high levels of tumor necrosis factor α have been found in sera[16,17] and nasal tissue[18] of ML patients, which may account for the increasing number of PMN neutrophils in nasal secretions. A switch of the predominant cell type from neutrophils to epithelial cells after treatment in those responding to therapy favors this hypothesis.

We have shown previously that upper respiratory tract infections may lead to an increase in the total number of cells and in the proportion of PMN neutrophils recovered in nasal lavages.[8] This observation raises concern about the interpretation of any findings during the follow-up of ML patients. An elevated cell count at 1 time point associated with symptoms of an acute respiratory infection should not be taken as an index of absence of response to antimony treatment in patients with ML.

Our findings on QNCs of ML subjects do not support the use of this method for diagnosis. Nevertheless, the association of a high total number of cells, and PMN predominance, with the presence of ML active lesions provides the grounds for its utilization as a marker of disease activity and an indicator of healing. Obtaining a total cell count by QNCs is simple enough to be performed in field studies, and a bilateral differential cytology may be obtained by nasal swabs replacing cytocentrifugation. A quantitative method of assessing ML activity, such as QNCs, is desirable, for clinical follow-up in areas lacking specialists in otolaryngology, and even more, as an inexpensive research tool.

ACKNOWLEDGMENT — We are grateful to E. Lago and his team in Corte de Pedra, Bahia, for their technical support in the field.

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EUROPEAN ACADEMY OF FACIAL PLASTIC SURGERY

The annual meeting of the European Academy of Facial Plastic Surgery will be held September 15-17, 2000, in Thessaloniki, Greece. For information, contact Prof Dr J. Daniilidis, ENT Dept, Aristotelian University of Thessaloniki-AHEPA Hospital, 540 06 Thessaloniki, Greece; telephone 0030/31/994.761-2; fax 0030/31/994.916.

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CHANGES IN EPIDERMAL GROWTH FACTOR RECEPTORS IN OLFACTORY EPITHELIUM ASSOCIATED WITH AGING

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To clarify the mechanisms of age-related changes in the olfactory epithelium, we investigated age-related changes in epidermal growth factor receptors (EGFRs) in the epithelium. We examined 3 mice at each of the following stages: embryonal (embryonic days 14 and 16), neonatal (postnatal days 1 and 7), adult (postnatal weeks 5 and 12), and aged (postnatal years 1.5 to 2). The olfactory epithelium of each mouse was stained with sheep anti-human EGFR polyclonal IgG by an immunohistochemical method. The EGFRs were observed in all layers of the olfactory epithelium at the embryonal and neonatal stages. They were identified only in the basal layer of the olfactory epithelium at adult and aged stages, and the number of regions in which EGFRs were identified in the basal layer of the olfactory epithelium decreased in aged mice compared to adult mice. We believe that a decrease in EGFRs in the olfactory epithelium induces the inhibition of cell proliferation, with resultant atrophy of the olfactory epithelium.

KEY WORDS — aging, epidermal growth factor receptors, immunohistochemical method, mouse, olfactory epithelium.

INTRODUCTION

The olfactory epithelium is unique in its ability to give rise to new sensory neurons throughout life, and neurogenesis is greatly enhanced following injury to the epithelium, allowing for a rapid reconstitution of the damaged epithelium.[1] However, atrophy of the olfactory epithelium and deterioration of olfactory function with aging have been reported.[2-4] We believe that the deterioration of olfactory function derives from atrophy of the olfactory epithelium that occurs with aging, and that this atrophy is related to a decrease in cell proliferation.

On the other hand, epidermal growth factors (EGFs) and transforming growth factor–α (TGF-α) are polypeptides that stimulate cell proliferation.[5,6] Farbman et al[7] demonstrated that EGFs and TGF-α promoted a substantial increase in the rate of cell division in a study on organ cultures of fetal rat olfactory mucosa. Accordingly, we believe that olfactory epithelium turnover is related to EGFs and TGF-α and their receptors, the epidermal growth factor receptors (EGFRs). For this reason, we examined the expression of EGFRs in the olfactory epithelia of mice, using an immunohistochemical method, and investigated the changes in EGFRs in the olfactory epithelia of embryonal, neonatal, adult, and aged mice.

MATERIALS AND METHODS

In order to elucidate their normal developmental changes, we examined the mice at the following stages: embryonal (embryonic days 14 and 16), neonatal (postnatal days 1 and 7), adult (postnatal weeks 5 and 12), and aged (postnatal years 1.5 to 2). We examined 3 mice at each stage. After fixation, each mouse was decapitated and the specimens were decalcified in 10% ethylenediaminetetraacetic acid.

The fixed specimens were embedded in paraffin and cut into 4-μm-thick sections. After microwave treatment, the sections were incubated for 24 hours at 4°C with sheep anti-human EGFR polyclonal antibody as the primary antibody. Immunohistochemical staining was performed with a Histofine kit (Nichirei, Tokyo, Japan). Immunoreactive EGFRs were visualized with diaminobenzidine and hematoxylin.

RESULTS

Before conducting this study, we confirmed the efficacy of the antibodies. In murine skin, sebaceous glands and follicle plugs were stained by anti-EGFR polyclonal antibody. For the negative controls, the primary antibodies were replaced with normal sheep serum. Neither the murine skin nor the murine olfactory mucosa was stained.

In the embryonic olfactory mucosa, the whole olfactory epithelium and mesenchyme were stained by anti-EGFR polyclonal IgG. Figure 1 shows the olfactory mucosa of embryonic day 16 after staining by anti-EGFR polyclonal IgG.

Neonatal specimens showed the same staining patterns as the embryonal specimens. Figure 2 presents the olfactory mucosa of a postnatal day 7 mouse.

The EGFRs were identified only in the basal layer of the olfactory epithelium at adult and aged stages, and the number of regions in which EGFRs were

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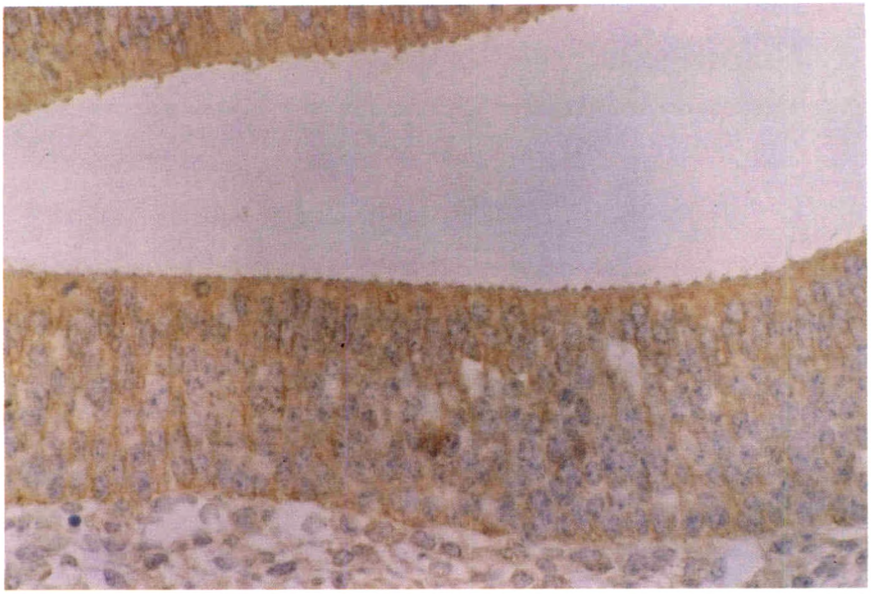

Fig 1. Olfactory mucosa of embryonic day 16 mouse stained by anti–epidermal growth factor receptor (EGFR) polyclonal IgG (diaminobenzidine and hematoxylin, original ×200). EGFRs were observed in all layers of olfactory epithelium.

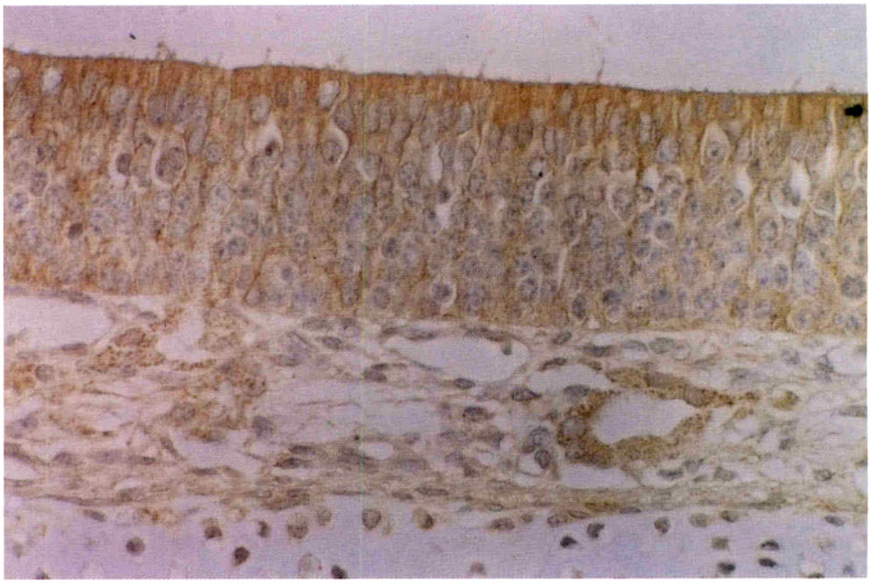

Fig 2. Olfactory mucosa of postnatal day 7 mouse stained by anti-EGFR polyclonal IgG (diaminobenzidine and hematoxylin, original ×200). EGFRs were observed in all layers of olfactory epithelium.

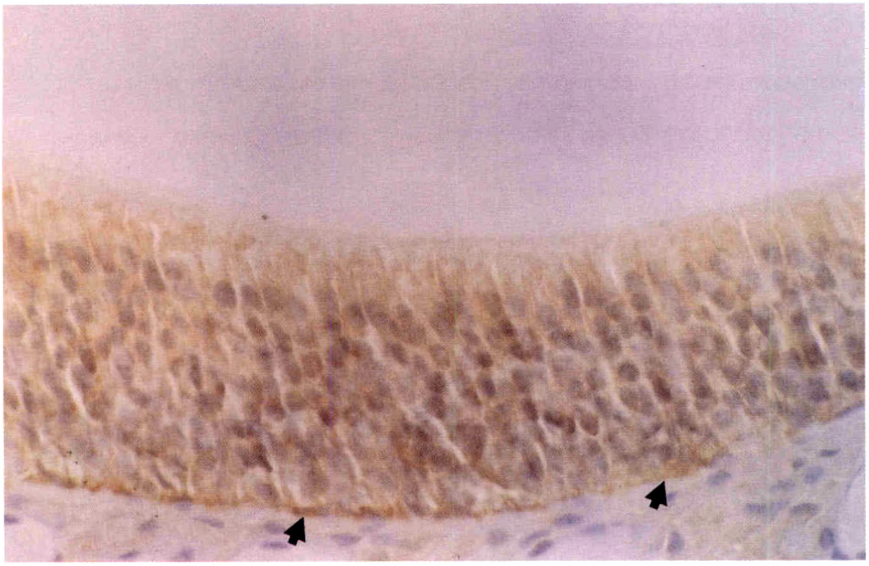

Fig 3. Olfactory mucosa of postnatal week 12 (adult) mouse stained by anti-EGFR polyclonal IgG (diaminobenzidine and hematoxylin, original ×200). EGFRs were identified in basal layer of olfactory epithelium (arrows).

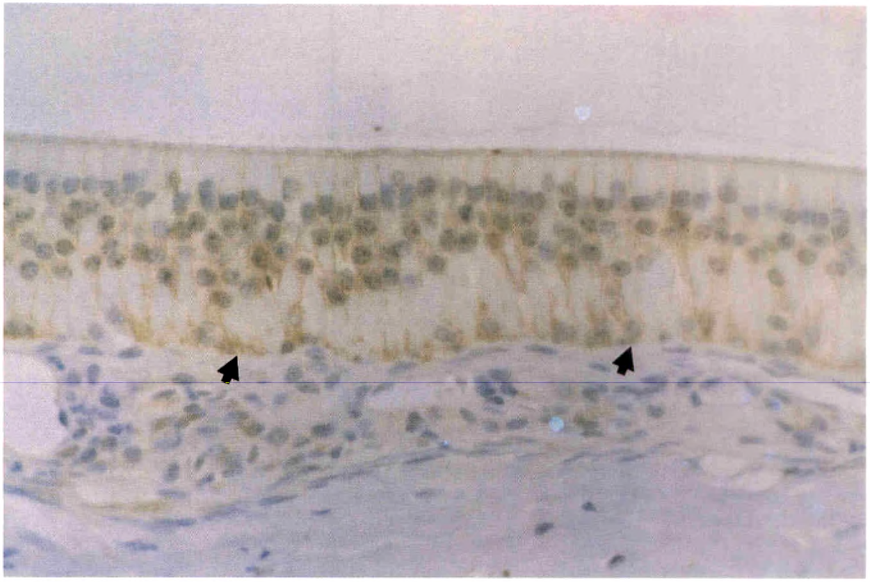

Fig 4. Olfactory mucosa of postnatal year 2 (aged) mouse stained by anti-EGFR polyclonal IgG (diaminobenzidine and hematoxylin, original ×200). EGFRs were identified in basal layer of olfactory epithelium, but only in certain regions (arrows). Note also significantly diminished thickness of mucosa.

identified in the basal layer of the olfactory epithelium decreased in aged mice compared to adult mice. Figure 3 shows the olfactory mucosa of an adult mouse, and Fig 4 shows the olfactory mucosa of an aged mouse after staining by anti-EGFR polyclonal IgG.

These changes in EGFRs were recognized in all 3 mice in each group.

The Table summarizes the results of staining by anti-EGFR polyclonal IgG.

DISCUSSION

The staining in the murine skin, sebaceous glands, and follicle plugs by anti-EGFR polyclonal antibody, and the lack of staining in the murine skin and murine olfactory mucosa by normal sheep serum, indicates that these antibodies were appropriate for the present study.[8,9]

We observed EGFRs in all layers of the olfactory epithelium in the embryonal and neonatal mice. We believe that EGFRs contribute to cell proliferation and growth of the epithelium at these stages.

EGFR EXPRESSION IN MOUSE OLFACTORY EPITHELIUM

Layer of Epithelium	*E1*	*E2*	*D1*	*D7*	*W7*	*W12*	*Y1.5-2*
Surface	++	++	++	+	±	–	–
Intermediate	++	++	+	±	±	–	–
Basal	++	++	++	++	+	+	±

EGFR — epidermal growth factor receptor; E — embryonic day; D — postnatal day; W — postnatal week; Y — postnatal year; ++ — strong; + — moderate; ± — some areas; – — absent.

On the other hand, EGFRs were identified only in the basal layer of the olfactory epithelium at adult and aged stages. The quantity of EGFRs at the adult and aged stages was clearly smaller than that at the embryonal and neonatal stages. Also, the number of regions in which EGFRs were identified in the basal layer of the olfactory epithelium decreased in aged mice compared to adult mice. This result suggests that EGFR expression in mouse olfactory epithelium appears to decrease continually with age.

Weiler and Farbman[10] examined the expression of proliferating cells in the olfactory epithelium of rats using an immunohistochemical method with anti-5-bromo-2'-deoxyuridine antibody to elucidate the changes in proliferating cells with aging. The number of proliferating cells in the olfactory epithelium decreased rapidly after birth.[10]

These changes in proliferating cells, which seem to result in atrophy of the olfactory epithelium, coincide with changes in EGFRs in the olfactory epithelia of mice.

In conclusion, the present data suggest that EGFRs are strongly related to cell proliferation and to growth and turnover of the olfactory epithelium. Further, a decrease in EGFRs may lead to inhibition of cell proliferation.

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INTERNATIONAL CONFERENCE ON NEWBORN HEARING SCREENING DIAGNOSIS AND INTERVENTION

The International Conference on Newborn Hearing Screening Diagnosis and Intervention will be held in Milan, Italy, October 12-14, 2000. In Europe, contact Dr Ferdinando Grandori, Center of Biomedical Engineering, Polytechnic of Milan, piazza Leonardo da Vinci 32, I-20133 Milan, Italy; fax +39.02.2399.3360; e-mail NHS2000@biomed.polimi.it. In the United States, contact Dr Deborah Hayes, The Children's Hospital, 1056 E 19th Ave, B030, Denver, CO 80218; telephone (303) 861-6424; fax (303) 764-8220; e-mail: Hayes.deborah@tchden.org.

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NASOPHARYNGEAL TONSIL'S PROVISION OF THE SURFACE SECRETIONS WITH IMMUNOCYTES, A PROPERTY ADDITIONAL TO ANTIGEN PROCESSING

MAGNUS IVARSSON, MD CHRISTER LUNDBERG, MD, PHD

GÖTEBORG, SWEDEN

As we recently found that IgA, IgM, and IgG are produced and secreted by immunocytes present in nasopharyngeal secretions, we tested the hypothesis that B- and T-lymphocytes in the surface secretions are derived from the nasopharyngeal tonsil in an active process. By immunohistochemistry, we found that numerous B- and T-lymphocytes were often accumulated in restricted areas in the epithelium, and some of these cells were demonstrated just beneath the epithelial surface or could be observed protruding into the lumen. A portion of these cells were Ki-67$^+$, indicating clonal expansion and/or immunoglobulin class switching. Analyses of the surface secretions by immunocytochemistry also demonstrated B- and T-lymphocytes, as well as Ki-67$^+$ cells — a finding that indicates that immunologically active cells are transported into the surface secretions. The results imply that there is a substantial migration from the nasopharyngeal tonsil of immunologically active cells into the surface secretions.

KEY WORDS — adenoid, immunoglobulins, mucosal immunity, nasopharynx, tonsil.

INTRODUCTION

While studying the defense system of the upper airway mucosa, we recently found that IgA, IgM, and IgG are produced, as well as secreted, by immunocytes present in nasopharyngeal secretions (unpublished observations). Considering the numbers of these immunocytes and the amount of immunoglobulin that can be produced by a single immunocyte, we concluded that the cell populations in the secretions might produce half of the immunoglobulins present in the secretions on the surface of the nasopharyngeal tonsil (adenoid).[1-3] This implies that the production of immunoglobulins in the secretions is, in all probability, of physiological importance. Consequently, the origin and the kinetics of the immunocytes in the secretions become essential issues to explore.

Several studies have revealed the presence of B-lymphocytes and plasma cells in the mucosal epithelium and the area just below the basement membrane.[4-10] These cells produce polymer immunoglobulins that are transported by the poly-Ig receptor into the surface secretions. A portion of the immunoglobulins are monomers, and they reach the mucosal surface by simple diffusion.[11-14] These immunoglobulins in the secretions are considered to be locally produced and are often claimed to explain the differences between concentrations of immunoglobulins in plasma and in secretions. The significance of immunoglobulins that are produced in the secretions by immunocytes has, to our knowledge, not been explored until now.

The present study was undertaken to test the hypothesis that B-lymphocytes and T-lymphocytes in the surface secretions on the nasopharyngeal tonsil are derived from the nasopharyngeal tonsil in an active process.

MATERIAL AND METHODS

The study was approved by the Ethics Committee of the Medical Faculty at Göteborg University. Informed consent was obtained from the parents of the children.

Collection of Specimens. Ten children, 8 boys and 2 girls, 3 to 8 years of age (median 5 years), participated in the study. They were scheduled for adenoidectomy due to secretory otitis media (SOM) and/or nasal obstruction. The children showed no signs of airway infection at the time of surgery.

Samples of the surface secretions were obtained from all children by a previously described imprint method.[15] Briefly, under general anesthesia and before the adenoidectomy, the soft palate was lifted and a plastic foam sponge (15 × 10 × 15 mm) was lightly pressed against the surface of the nasopharyngeal tonsil and then immediately pressed against a glass slide. The imprints were air-dried and fixed in ice-chilled acetone for 5 minutes, then stored at −20°C until further analyses, performed within a week. After the imprints had been obtained, the nasopharyngeal tonsil was removed, and samples of the

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nasopharyngeal tissue were fixed in 4% formaldehyde and embedded in paraffin.

Test of Imprint Pressure. To determine the pressure applied to the surface of the nasopharyngeal tonsil by the plastic foam sponge as imprints were being obtained, the following test was performed. A plastic foam sponge (15 × 10 × 15 mm) held in surgical forceps, as in the normal sampling situation, was pressed against a scale (Mettler PM600) until the sponge was almost completely compressed. This procedure was repeated 10 times, and the pressure was registered as weight in grams on the scale.

Primary Antibodies Used in Staining of Imprints and Tissue Sections. The following antibodies were selected: CD79a for identification of B-lymphocytes (CD79a, JCB117), VS38c for identification of plasma cells (DAKO plasma cell, VS38c), CD3 for detection of T-lymphocytes (T-cell, CD3), CD4 for identification of T-helper cells (T-cell, CD4, MT310), CD8 for identification of T-suppressor cells (T-cell, CD8, C8/144B), and Ki-67 for identification of proliferating cells (Ki-67 antigen). All primary antibodies were purchased from DAKO, Glostrup, Denmark.

Immunocytochemical Staining of Imprints. The acetone-fixed imprints were allowed to stand at room temperature for 2 to 3 hours and were stained according to the manufacturer's directions, with slight modifications. All imprints were washed in Tris-buffered saline (TBS) between incubations and placed in a moist chamber during incubations. Briefly, the imprints that were to be stained with antibodies directed against Ki-67 or CD3 were incubated with 3% hydrogen peroxide for 5 minutes. Thereafter, all imprints were blocked with TBS containing 1% bovine serum albumin (BSA) for 20 minutes. Excess BSA was removed, and the imprints were exposed to monoclonal mouse antibodies directed against CD4 or CD8, or to polyclonal rabbit antibodies directed against Ki-67. One imprint from each child was double-stained and exposed to both polyclonal rabbit antibodies directed against CD3 and monoclonal mouse antibodies directed against CD79a. All antibodies were diluted 1:100 in TBS containing 1% BSA, except CD4 (1:20) and CD8 (1:50). The imprints were incubated overnight at 4°C. Thereafter, the imprints stained with CD79a, CD4, or CD8 antibodies were incubated with the secondary antibody (E433, biotinylated goat anti-mouse immunoglobulins, DAKO) for 40 minutes at room temperature. The imprints stained with Ki-67 or CD3 antibodies were incubated with goat anti-rabbit antibodies (Envision+, horseradish peroxidase, DAKO) for 30 minutes, and then developed by exposure to diaminobenzidine (DAB, DAKO) solution for 8 to 10 minutes. The imprints stained with CD79a, CD4, or CD8 antibodies were exposed to streptavidin-conjugated alkaline phosphatase (BioGenex Laboratories, San Ramon, Calif) and incubated for 40 minutes at room temperature.

The imprints were then developed by exposure to 4-chloro-2-methylbenzenediazonium salt (FastRed, BioGenex Laboratories) for 4 to 5 minutes. All imprints were counterstained with Mayer's hematoxylin for 2 minutes and were then rinsed in running water for 10 minutes prior to mounting. As a negative control for the CD79a, CD4, and CD8 antibodies, the primary monoclonal mouse antibody was replaced by another monoclonal mouse antibody (IgG1, DAKO), and as a negative control for Ki-67 and CD3, the primary polyclonal rabbit antibody was replaced by rabbit immunoglobulins (DAKO). The imprints were examined under a light microscope (Optiphot, Nikon).

Immunohistochemical Staining of Tissue Sections. The serial sections, 5 µm thick, of paraffin-embedded nasopharyngeal tonsils were deparaffinized and rehydrated with target retrieval solution (S 1700, DAKO) according to the manufacturer's directions. All sections were washed in TBS between incubations and placed in a moist chamber during incubations.

The sections to be stained with antibodies directed against Ki-67 or CD3 were incubated with 3% hydrogen peroxide for 5 minutes in order to inhibit the endogenous peroxidase activity. In the sections to be stained with CD79a, VS38c, or CD8 antibodies, the endogenous biotin was inhibited (biotin-blocking system, DAKO). Next, the sections were blocked by adding 1% BSA diluted in TBS for 20 minutes. Excess BSA was removed, and the sections were exposed to monoclonal mouse antibodies directed against CD79a, VS38c, or CD8, or to polyclonal rabbit antibodies directed against CD3 or Ki-67. All antibodies were diluted 1:100 in TBS containing 1% BSA, except CD8, which was diluted 1:50, and were incubated overnight at 4°C. Then the CD79a, VS38c, and CD8 sections were incubated with rabbit anti-mouse immunoglobulins (E413, DAKO) for 40 minutes at room temperature. The Ki-67 and CD3 sections were exposed to goat anti-rabbit antibodies (Envision+, horseradish peroxidase, DAKO) for 30 minutes and developed by exposure to DAB for 8 to 10 minutes.

The CD79a, VS38c, and CD8 sections were incubated for 20 minutes with streptavidin complexed with biotinylated alkaline phosphatase (StreptAB-Complex/AP, DAKO) and then developed by exposure to a New Fuchsin substrate system (DAKO) for 8 minutes. All sections were counterstained with Mayer's hematoxylin for 1 minute, rinsed in running water, and mounted. As a positive control for the immunostaining of plasma cells, we used formalin-fixed nasal polyps, which are known to be enriched with plasma cells. As a negative control for the CD79a, CD4, and CD8 antibodies, the primary monoclonal mouse antibody was replaced by another monoclonal mouse antibody (IgG1, DAKO), and as a negative control for Ki-67 and CD3, the primary polyclonal rabbit antibody was replaced by rabbit immunoglobulins (DAKO). The sections were examined under a light microscope (Optiphot, Nikon).

Quantification of Cells in Imprints. In our rating system, "occasional" corresponds to 1 to 10 cells in the whole imprint, "sparse" corresponds to 1 to 5 cells in every second or third field of vision (200×), and "abundant" corresponds to >10 cells in every field of vision (200×).

Quantification of Cells in Sections. "Sparse" corresponds to 1 to 5 cells in every field of vision (200×), "moderate" corresponds to 6 to 20 cells in every field of vision (200×), and "abundant" corresponds to >20 cells in every field of vision (200×).

RESULTS

Imprint Pressure. The pressure applied by the sponge during imprint, registered as weight in grams on the scale, varied between 56 and 84 g, with a mean of 76 g (data not shown). The latter value equals a pressure of 50 cm H_2O/cm^2.

Imprints. May-Grünewald-Giemsa staining revealed an invariable presence but various distributions and amounts of epithelial cells and mononuclear and polymorphonuclear leukocytes in the imprints of all patients. The cells in the imprints were unevenly distributed and often accumulated in groups. The epithelial cells were discriminated from leukocytes by size and morphology. Bacteria, free or adhered to squamous epithelial cells, were observed in all imprints, as were bacteria surrounded by granulocytes, indicating active phagocytosis.

The double staining with CD79a and CD3 entailed technical problems, as the brown staining established by the peroxidase technique often markedly quenched the red staining established by the biotin-avidin technique. However, $CD3^+$ T-lymphocytes and $CD79a^+$ B-lymphocytes were demonstrated in all imprints, with a dominance of T-lymphocytes. The number of B-lymphocytes and T-lymphocytes in the imprints showed considerable interindividual variation and ranged from sparse to abundant. Some of the B-lymphocytes and T-lymphocytes were seen in contact with each other or within a distance of less than 1 cell-width.

$CD4^+$ T-lymphocytes were present in imprints from all patients (Fig 1A). The number of positive cells showed considerable interindividual variation, and the number of cells varied from occasional to abundant. The cells appeared singly or as 2 or 3 cells together. The $CD4^+$ cells were often seen in contact with other mononuclear cells.

$CD8^+$ T-lymphocytes were present in imprints from all but 2 patients (Fig 1B). The number of cells, in the imprints with positive cells, varied from sparse to abundant and, in all but 2 imprints, was estimated to be less than the number of $CD4^+$ T-lymphocytes present in the corresponding CD4-stained imprints. The cells appeared singly or as 2 or 3 cells together. The $CD8^+$ cells were often seen in contact with other mononuclear cells.

$Ki\text{-}67^+$ cells were present in imprints from all patients (Fig 1C). The number of stained cells showed interindividual variation, ranging from sparse to abundant.

Tissue Sections. The characteristic architecture of the nasopharyngeal tonsil, including epithelium, mucosal crypts, germinal centers, mantle zones, extrafollicular regions, and small blood vessels just beneath the basal membrane, was observed in all sections. In the crypts, the epithelium was mainly reticulated. The surface epithelium in the mucosal areas directed toward the nasopharyngeal lumen was alternately ciliated or squamous in character. The ciliated epithelium and squamous epithelium varied in dominance between the different specimens. Infiltration of B-lymphocytes was seen in both types of epithelium in all specimens, but to various extents (Fig 2). In some specimens, only few and small areas were infiltrated, whereas in other specimens, many and large areas of the epithelium were infiltrated by B-lymphocytes. The number of infiltrating B-lymphocytes also varied intra-individually and interindividually from sparse to abundant. In areas with heavy B-lymphocyte infiltration, the epithelial cells were often separated by the B-lymphocytes, indicating broken tight junctions. Sometimes the B-lymphocytes were gathered in the epithelium, as in established compartments. In all specimens, various numbers of B-lymphocytes were seen immediately under the mucosal surface. The epithelial cells were occasionally separated from each other at the surface, forming a narrow channel with 1 or a few B-lymphocytes in the immediate vicinity. B-lymphocytes virtually passing out into the lumen were observed in all specimens. In many areas with extensive infiltration of B-lymphocytes in the epithelium, the basement membrane could not be observed with the morphological staining used.

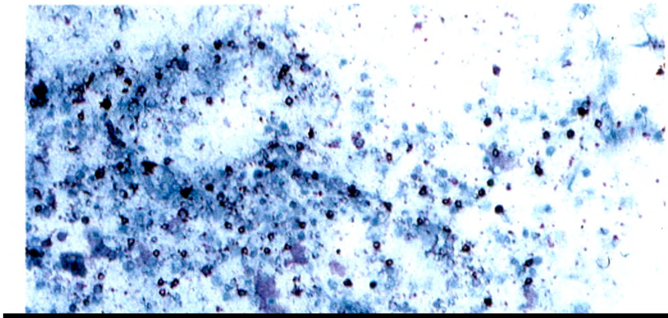

Fig 1. Surface secretions from nasopharyngeal tonsils obtained by imprint technique (original ×200). A) Immunocytochemical staining demonstrating CD4+ T-lymphocytes (red cells). B) Immunocytochemical staining demonstrating CD8+ T-lymphocytes (red cells). C) Staining with antibodies directed against Ki-67 antigen demonstrating proliferating cells (dark cells).

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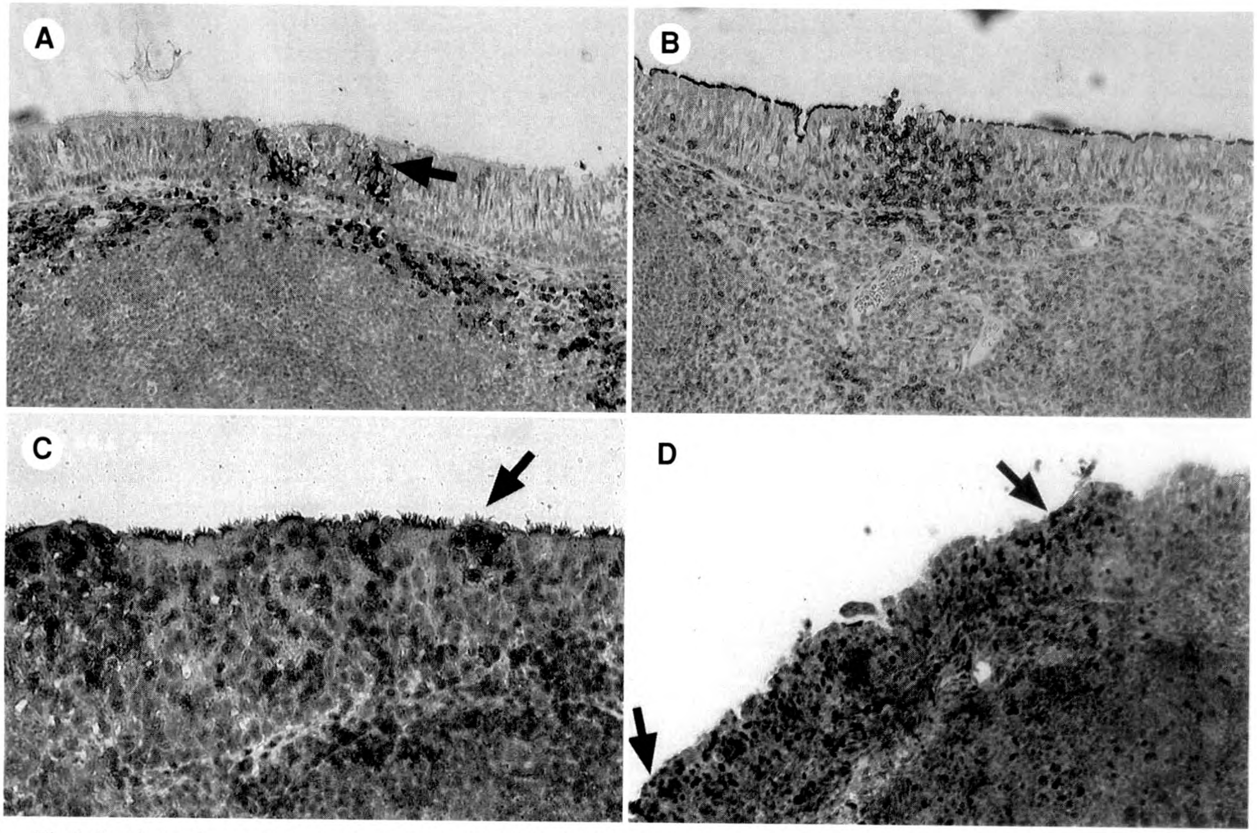

Fig 3. Sections of nasopharyngeal tonsils. A) Stained with VS38c antibodies demonstrating plasma cells (dark cells; original ×200). Plasma cells penetrate intact ciliated epithelium, and some cells reach luminal surface (arrow). Numerous plasma cells are present below basement membrane. B) Stained with CD3 antibodies demonstrating T-lymphocytes (dark cells; original ×200). T-lymphocytes are observed scattered in ciliated epithelium and also accumulated in restricted area. Some cells penetrate luminal surface. C) Stained with CD8 antibodies demonstrating $CD8^+$ T-lymphocytes (dark cells; original ×400). $CD8^+$ cells are present in intact ciliated epithelium, and some cells are observed just below epithelial surface (arrow). D) Stained with antibodies directed against Ki-67 antigen demonstrating proliferating cells (original ×200). Positive cells are observed as dark cells in epithelium, and some cells protrude into lumen (arrows).

to the surface, and some were also seen protruding into the nasopharyngeal lumen (Fig 3B).

$CD8^+$ cells could invariably be demonstrated in the epithelium of all specimens. The number of positive cells in the epithelium ranged from sparse to moderate, but was estimated to be fewer than the number of $CD3^+$ cells in the epithelium from the same specimen. $CD8^+$ cells could be demonstrated close to the surface in all specimens, and some cells could be observed protruding into the nasopharyngeal lumen (Fig 3C).

Cells stained by the Ki-67 antibody were invariably seen in the epithelium, and the number ranged from sparse to abundant. In the areas with accumulated B-lymphocytes, the number of $Ki\text{-}67^+$ cells was often enhanced. In areas with squamous epithelium, in particular, sparse to moderate numbers of positive cells were present close to the basement membrane. However, in areas with B-lymphocyte accumulations, $Ki\text{-}67^+$ cells were always present close to the surface, and some cells were protruding into the nasopharyngeal lumen (Fig 3D).

In the reticulated epithelium in the crypts, B-lymphocytes, $CD3^+$ and $CD8^+$ T-lymphocytes, plasma cells, and $Ki\text{-}67^+$ cells were routinely observed and were often present in the immediate vicinity of the surface, and some cells protruded into the crypt lumen.

Six patients suffered from SOM, 2 suffered from nasal obstruction, and 2 had a history of both SOM and nasal obstruction. There were no correlations between the studied parameters in the tissue specimens and the imprints, respectively, and the case histories of the children.

DISCUSSION

This study was undertaken to determine whether lymphocytes migrate from the nasopharyngeal tonsil into the surface secretions, and if so, whether this activity can be considered to be part of an active process.

The children in the study suffered from SOM and/or snoring. In all other respects, they were healthy and, at the time of surgery, free from clinical signs of infection. We therefore considered them to be normal, healthy children, and that our results would have a rather universal applicability.

As the secretions on the nasopharyngeal tonsil form a thin film, sampling was performed by an imprint technique. This technique allows sampling from a large mucosal area and maintains, in the imprint, the spatial relations cells have in the secretions in vivo.[15]

When speaking and swallowing, children with a large nasopharyngeal tonsil press the soft palate against this tonsil. In children, data are lacking, but in adults, the velopharyngeal closure force during speech is about the same (and during swallowing, much higher) as compared to the pressure applied to the surface of the nasopharyngeal tonsil with the imprint technique: mean 50 cm H_2O/cm^2.[16] The sampling procedure can therefore not be expected to harm the epithelial surface or cause artifacts in the imprints. Further, the lack of red blood corpuscles, or the presence of only scant numbers of them, in the imprints implies that the sampling caused no damage to the mucosa.

The antibody used for identification of plasma cells, VS38c (DAKO), also stains epithelial cells.[17] However, by the distinct staining of the plasma cells, together with their morphology,[18] we could distinguish them from the epithelial cells.

Our study revealed that B- and T-lymphocytes and plasma cells, as also shown by others, were present in the epithelium of the mucosa of the nasopharyngeal tonsil.[4-10,19] In addition, we observed that these cells were not uniformly infiltrating the epithelium, but were accumulated in rather distinct epithelial areas, from which lymphocytes also protruded into the lumen. The cells infiltrated and passed through both ciliated and squamous epithelia. The finding that the accumulation and migration of the cells into the lumen were restricted to rather demarcated areas and not confined to any particular type of surface epithelium supports the notion that the lymphocytes migrate into the surface secretions as part of an active and locally regulated process. This implies that the relative distribution of ciliated and squamous surface epithelia[20] does not influence the possibility of the nasopharyngeal tonsil's providing the surface secretions with immunologically active cells.

Plasma cells were almost exclusively present in the areas of the epithelium in which the B-lymphocytes were accumulated, but, like the T-lymphocytes, were in smaller numbers than the B-lymphocytes. In these areas, substantial numbers of cells were often Ki-67$^+$, indicating proliferation. Although some of the cells, particularly those close to the basement membrane, may be proliferating epithelial cells, most of the cells were in all probability young and clonally expanding B- and T-lymphocytes. However, B-lymphocytes that change production of immunoglobulins — from, for example, IgM to IgA (isotype switching) — may also express Ki-67.[21] Again, the presence of Ki-67$^+$ mononuclear cells in the secretions is evidence for the notion that the Ki-67$^+$ cells in the epithelial layer of the corresponding sections are mainly lymphocytes and not proliferating epithelial cells. This finding indicates that there is immunologic activity in these restricted areas in the mucosal epithelium of the nasopharyngeal tonsil.

In all patients, B-lymphocytes, T-lymphocytes, and Ki-67$^+$ cells could regularly be observed in the imprints. As these types of cells are the same as those present in the epithelial layer in sections of the corresponding nasopharyngeal tonsils, the observations support the hypothesis that the cells in the secretions emanate from the nasopharyngeal tonsil. A tendency toward a correlation could be seen between the number of lymphocytes in the epithelial layer of the studied sections and the number of the same types of cells in the corresponding imprints. However, the variations within each imprint and each section were mostly so great that it seems reasonable to claim that the variability is due to restricted local conditions and that there are no uniform reactions in the mucosa of the nasopharyngeal tonsil as a whole.

We regularly found both CD4$^+$ T-lymphocytes and CD8$^+$ T-lymphocytes in the surface secretions. There was great intra-individual and inter-individual variation, but mostly, the CD4$^+$ T-lymphocytes predominated. Taken together, these observations of cellular variation are further evidence that the lymphocytes in the secretions fulfill an immunologic function in the secretions. This statement is also supported by our recent observation that immunocytes in the secretions produce and secrete substantial amounts of IgA, IgM, and IgG (unpublished observations).

The lack of correlation between the studied cellular parameters in imprints and tissue sections, respectively, and the clinical diagnoses of the children may be explained by the low numbers of participating patients. However, a more plausible explanation is that the children were free from clinically manifested infection at the time of sampling.

In all tissue sections, several cells were observed protruding from the epithelial surface and migrating into the lumen. As the thickness of the sections was approximately 5 µm, about 2,000 or more sections could theoretically be obtained from a single nasopharyngeal tonsil. Considering these data, one can estimate that at the moment of sampling, some thousand lymphocytes were actually entering the secretions. Although surprisingly high, this figure is in accordance with our previous observation that up to 2 million lymphocytes were present in the surface secretions on the nasopharyngeal tonsil (unpublished observations). We can at present only speculate about the function of these large numbers of lymphocytes in the secretions. They may, for example, be part of the defense system of the nasopharyngeal cavity, modulating the micromilieu and the inflammatory and immunologic processes in this strategic region of the upper respiratory tract. Further, it was recently reported that on the apical part of the epithelial cells in human fetal and adult proximal intestines, there is a receptor for binding IgG, probably a neonatal Fc receptor, FcRn.[22] It is suggested that this receptor facilitates the transport of IgG antibodies from the mother's milk into the gut mucosa of a neonate, promoting passive immunity, induction of oral tolerance, and immunosurveillance. Maybe the immunoglobulins produced in the nasopharyngeal secretions have the same function as those in mother's milk.

In conclusion, there is a substantial migration of immunologically active cells into the surface secretions from the nasopharyngeal tonsil.

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IMAGING CASE STUDY OF THE MONTH

PEDIATRIC MANDIBULAR ANEURYSMAL BONE CYST

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INTRODUCTION

Aneurysmal bone cysts (ABCs) are uncommon benign lesions of unknown cause first described by Jaffe and Lichtenstein[1] in 1942. Although they radiologically resemble true cysts, ABCs actually represent pools of extravasated blood encased in reactive fibrous connective tissue without an epithelial lining. Typically, these lesions are solitary, well circumscribed, and expansile. Although the expanding mass often disrupts the normal bony architecture with erosion of the cortex, the tumor usually is covered by a thin band of periosteal new bone without extension into the adjacent soft tissues.[2]

Aneurysmal bone cysts occur most commonly in the long bones and vertebral bodies. Involvement of the craniofacial bones is rare and represents approximately 5% of all bone lesions occurring in that area. Due to their rarity, ABCs may be confused radiologically with neoplastic lesions that typically require more aggressive therapy.

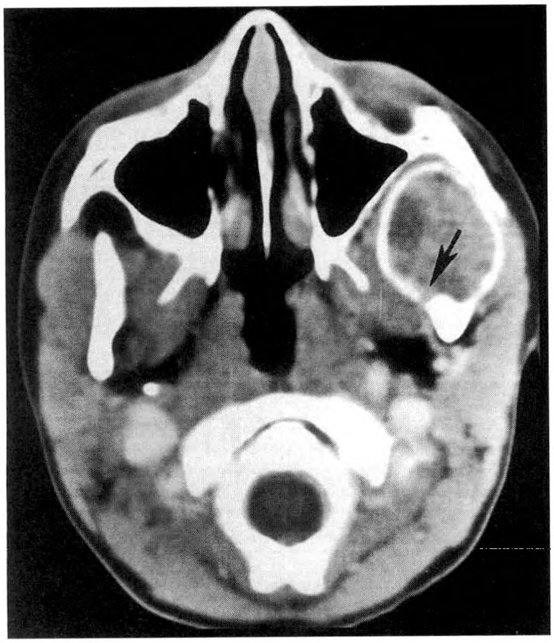

Fig 1. Axial contrast-enhanced computed tomographic image demonstrating heterogeneous expansile mass within left mandibular ramus. Overlying smooth, sclerotic bony rim shows small area of cortical erosion (arrow), but there is no extension into adjacent soft tissues.

CASE REPORT

An 8-year-old boy presented with a 5-day history of fullness and firmness of the left cheek that initially was noted by his mother shortly after a fall from his bicycle. The area was asymptomatic, without associated trismus, facial pain, weight loss, oral bleeding, or epistaxis. On physical examination, an obvious fullness was noted in the left preauricular region in the area of the left zygomatic arch. A thorough physical examination of the neck, nose, oral cavity, oropharynx, and hypopharynx as well as neurologic evaluation revealed findings within normal limits. The patient demonstrated a class I occlusion with no deviation upon opening. Intraoral palpation

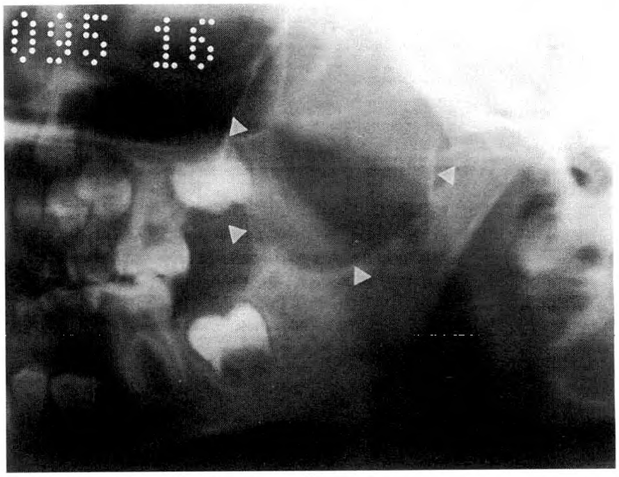

Fig 2. Close-up of panoramic radiograph demonstrating expansile mass thinning cortex of ramus and coronoid process (arrowheads).

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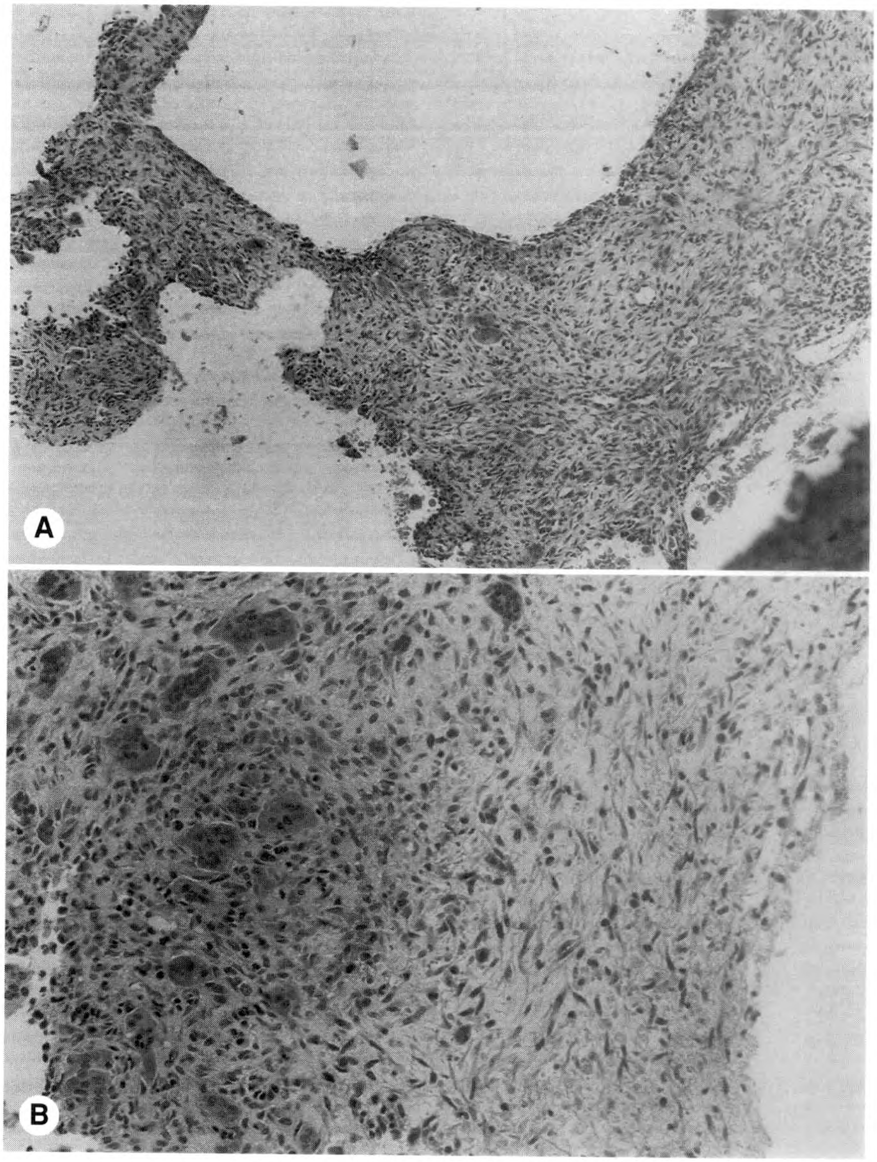

Fig 3. Photomicrographs of surgical specimen. A) Demonstrating cystlike structures (original ×100). B) Demonstrating scattered osteoclastic multinucleated giant cells (original ×400).

of the left proximal mandible revealed a firm, minimally tender bony expansion that measured approximately 5 × 5 cm.

Radiologic evaluation included a computed tomography scan that demonstrated a large, nonenhancing, expansile, lytic lesion of the mandible in the ramus and coronoid process (Fig 1). The periphery of the lesion was surrounded by a thin sclerotic rim of bone. Only a small focus of cortical perforation was present, and no extension into the surrounding soft tissue was evident. A panoramic radiograph depicted a radiolucent lesion extending from the retromolar area to the condylar neck with erosion of the coronoid process (Fig 2). The referring pediatric oncologist ordered a bone scan to evaluate the metabolic activity of this lesion and to rule out active bone lesions at other sites. No abnormal tracer uptake was observed at technetium 99 bone scintigraphy. Although malignancy could not be excluded, the radiologic pattern strongly suggested a benign lesion with ABC the primary consideration. Central giant cell granuloma, traumatic bone cyst, intraosseous hemangioma, and Langerhans' cell disease also were in the differential diagnosis.

A core needle biopsy revealed rare multinucleated giant cells and numerous histiocytes, some of which contained hemosiderin. Without evidence of malignancy, the lesion was treated by thorough curettage and cautery. Intraoperatively, the lesion presented as a fibrous-walled cystic lesion arising within the coronoid process of the mandible.

Histopathologic examination of the surgical specimen revealed numerous pieces of a cystlike structure that was devoid of any organized epithelial lining and consisted of reactive and cellular fibrous connective tissue (Fig 3A). Scattered individual sites and clusters of osteoclastic multinucleated giant cells were noted (Fig 3B). Occasional trabeculae of vital bone and smaller intact blood lakes were seen within the fibrous connective tissue wall. The final histopathologic diagnosis was cystic fibro-osseous lesion with giant cells, consistent with ABC.

The patient continues to return for periodic clinical and radiographic evaluation and has demonstrated no evidence of recurrence 3 years after surgery.

DISCUSSION

Several divergent theories of the pathogenesis of ABCs exist, with no one cause receiving dominant support. Although the exact cause is indeterminate, these lesions are considered reactive and may arise de novo or intermixed with preexisting bony lesions such as fibrous dysplasia, osseous dysplasia, giant cell tumor, granuloma, chondroblastoma, and osteoblastoma.[3]

According to Giddings et al,[4] 70% of ABCs occur in the vertebrae and long bones such as the tibia, humerus, and femur. Less commonly, ABCs also may involve the skull and maxillofacial region. Two percent of all cases arise in the jaws,[2] with 60% of these occurring in the mandible.[4] The first mandibular ABC was reported by Sherman and Soong[5] in 1957. In general, the peak prevalence of ABCs is during the second and third decades of life, with approximately 90% occurring prior to 30 years of age. Although no race predilection is noted, a slight female predominance has been documented in some investigations.

Definitive diagnosis of ABC requires histopathologic confirmation with clinical and radiologic correlation. Clinically, the lesions usually present as firm, nonpulsatile swellings that are well localized and without bruit upon auscultation. Pain rarely is reported except in those cases that impinge on an adjacent nerve or joint capsule.[2] Although the radiologic features are not diagnostic, they are useful in establishing the differential diagnosis and assessing postoperative resolution. Radiographs such as panoramic radiography or computed tomography scans typically reveal a unilocular or multilocular cystic lesion that often is surrounded by a thin but distinct rim of periosteal new bone. This "soap bubble" appearance may be present due to resorption of cancellous bone and replacement with blood. Magnetic resonance scanning will show the nonspecific but common finding of "fluid-fluid levels."[6] In addition, magnetic resonance imaging may provide better demonstration of the lesion's extent if radiography is unclear.[7]

Histopathologically, ABCs consist of cavernous, blood-filled sinusoids, surrounded by reactive fibrous connective tissue, that usually contain hemosiderin-laden macrophages, osteoclastic giant cells, and trabeculae of reactive bone. Even though the clinical and radiographic features may suggest the diagnosis, Trent and Byl[2] reaffirm that histopathologic confirmation is necessary for the definitive diagnosis.

The routine management of ABCs of the jaws is complete curettage with or without desiccation, cryotherapy, or sclerosis. Lesions that significantly weaken the mandible and place it at high risk for pathological fracture may benefit from cancellous bone grafting, but this is usually not necessary, as the bone will regenerate spontaneously in most cases.[8] Recurrence of ABC following conservative therapy such as simple curettage is not rare, with reported recurrence rates as high as 59%.[9] Tillman et al[10] report higher recurrence rates in patients less than 15 years of age. When curettage was combined with cryosurgery, the recurrence rate decreased to 18%. Although irradiation can result in resolution of the lesion, the possibility of postirradiation osteogenic sarcoma makes this modality unacceptable. Due to the rate of recurrence reported, these patients should be followed at least biannually with serial examinations and panoramic radiographs. Since recurrence is rare more than 2 years postoperatively, longer follow-up may be unnecessary.[11]

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CLINICOPATHOLOGICAL CONSULTATION

PARANEOPLASTIC SYNDROMES IN PATIENTS WITH LARYNGEAL AND HYPOPHARYNGEAL CANCERS

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Paraneoplastic syndromes are systemic, nonmetastatic manifestations associated with a variety of malignant neoplasms and occurring in a minority of cancer patients. Paraneoplastic syndromes in association with cancers of the larynx and hypopharynx are considered rare. The world literature is reviewed, dividing paraneoplastic syndromes into 4 main groups, ie, cutaneous (acanthosis nigricans, Bazex's syndrome, Bowen's disease, bullous pemphigoid, dermatomyositis, pruritus, Sweet's syndrome, yellow nail syndrome, and tylosis), endocrine (carcinoid syndrome, adrenocorticotropic hormone syndrome, Schwartz-Bartter syndrome, hypercalcemia), hematologic (Trousseau's syndrome), and neurologic (cerebellar degeneration, Eaton-Lambert myasthenic syndrome). A paraneoplastic syndrome may precede the clinical manifestations of a persistent or recurrent tumor or of asymptomatic metastases, and its onset may even, in a minority of cases, be the presenting sign of a malignancy. Otolaryngologists and head and neck surgeons should have a knowledge of the paraneoplastic syndromes in order to facilitate their detection of malignancies or recurrences.

Cancer patients may present different signs and symptoms that are generally determined locally, regionally, and remotely by the location and size of the neoplastic lesion. In a minority of patients, the signs and symptoms are due to the presence of an evident or occult cancer, but are not directly related to the primary mass or its metastases, appearing rather to be associated with the tumor on the basis of other, as yet ill-understood mechanisms. These conditions are usually referred to as paraneoplastic syndromes, and their location does not coincide with the site of the tumor.

Small cell lung cancer is the most frequent cause of paraneoplastic syndromes, and a wide array of conditions are associated with this oncotype, though they may occur in almost all types of malignant neoplasm involving various organs.

The association of paraneoplastic syndromes with tumors of the larynx and hypopharynx has occasionally been reported. They are also called paraneoplastic conditions, paraneoplastic effects, nonmetastatic syndromes, paraneoplastic phenomena, paraneoplastic disturbances, or remote effects, and they may precede the clinical manifestations of a tumor (persistent or recurrent tumor) or of asymptomatic metastases, and their appearance may thus be the first sign of a malignancy in a minority of cancer patients. Paraneoplastic syndromes have been identified with increasing frequency because of a greater clinician awareness and the identification of specific autoantibodies for some syndromes — neurologic syndromes in particular.

Paraneoplastic syndromes associated with laryngeal and hypopharyngeal cancer can be divided into 4 main groups: paraneoplastic cutaneous or dermatologic syndromes; paraneoplastic endocrine syndromes; paraneoplastic hematologic syndromes; and paraneoplastic neurologic syndromes.

Different oncotypes, such as squamous cell carcinoma, typical carcinoid, atypical carcinoid, and small cell neuroendocrine carcinoma, have been associated with paraneoplastic syndromes in patients with cancer of the larynx and hypopharynx.

DERMATOLOGIC SYNDROMES

Dermatologic syndromes include a very long list of cutaneous lesions. Acanthosis nigricans, Bazex's syndrome, Bowen's disease, bullous pemphigoid, dermatomyositis, pruritus, Sweet's syndrome, yellow nail syndrome, and tylosis have been observed in patients with laryngeal and hypopharyngeal cancer.

Acanthosis nigricans is the most frequent paraneoplastic lesion of the skin. It is a cutaneous paraneoplastic syndrome characterized by the presence of symmetric, light or dark brown areas of hyperpigmentation with orthokeratosis, hyperkeratosis, exaggerated skin markings, and warty lesions, particularly involving the intertriginous and flexural areas, especially the axilla, posterior neck, anogenital region, umbilicus, and areola. However, widespread

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involvement of the hands, feet, and mucosal membranes has also been seen. The most frequent association is with abdominal cancers, and particularly gastric carcinomas, but the process has also been described in association with squamous cell carcinoma of the hypopharynx.[1,2]

Bazex's syndrome (also termed Bazex's acrokeratosis paraneoplastica, acrokeratosis paraneoplastica, acrokeratosis Bazex) was described in 1965[3] as a paraneoplastic process associated with a carcinoma of the pyriform sinus, but it had already been reported by Gougerot and Rupp[4] in 1922 in association with a squamous cell carcinoma of the tongue. The initial reports came mainly from the European literature, and from French authors in particular, but the lesion is now reported with increasing frequency all over the world.[5] This paraneoplastic dermatosis has been seen mainly in white men over the age of 40 years, and only a few cases have been documented in black men and women.[6-13]

Clinically, it is characterized by erythematous squamous cutaneous plaques that spread centripetally — a psoriasiform cutaneous eruption — with a predilection for the extremities (ears, nose, fingers, toes) and, less frequently, the elbows, knees, and trunk, when the neoplastic mass is detected with difficulty or persists untreated, and nail dystrophy. Sometimes, even vesicles, bullae, and scabs have been described, particularly on the fingers, hands, and feet. The distribution of the cutaneous manifestations is generally symmetric.

According to Bazex and Griffiths,[9] the skin lesions develop in 3 stages.[5,14,15] The first stage begins with a psoriasiform eruption confined to the fingers and toes, the bridge of the nose, and the helix of the ear. Nail plate destruction can occur. In the second stage, the eruption becomes more extensive: the palms of the hands and soles of the feet become erythematous and scaly, with a characteristic violaceous color. The erythema and scaling spread to involve the cheeks, and the external ear is affected. The third stage is characterized by involvement of the back of the hands, the elbows, knees, arms, thighs, legs, scalp, and trunk. The lesions are erythematous to violaceous, with a fine adherent scale.

Histologically, these lesions are nonspecific. The most common histologic features are orthokeratosis with parakeratotic foci, an irregularly acanthotic epidermis with an intact basal cell layer, and a predominantly perivascular lymphocytic infiltrate in the upper dermis. Less commonly reported histologic changes in the epidermis include psoriasiform acanthosis, focal dyskeratosis, vacuolar degeneration of the prickle cells, and focal liquefaction of the basal cells with an infiltrate at the dermoepidermal interface.[16]

To date, a total number of 113 cases of Bazex's syndrome have been reported in association with malignancies,[5,15,17-24] and nearly half of the tumors were located in the oral cavity, pharynx, and larynx.[23] In the past, this dermatosis was erroneously defined as a process associated with supradiaphragmatic malignancies. It occurs more commonly, however, in patients with a primary squamous cell carcinoma of the head and neck metastasizing to the cervical lymph nodes,[5] especially with a cancer of the oral cavity, pharynx, and larynx, with lung and esophageal cancers, and with squamous cell carcinomas of unknown primary site with cervical nodal involvement. However, this process has been related to malignancies occurring in the bronchus, thymus, prostate, uterus, stomach, liver, bladder, and vulva. This dermatologic condition is associated with various oncotypes (squamous cell carcinoma, adenocarcinoma, anaplastic carcinoma, neuroendocrine carcinomas, transitional cell carcinoma, myeloma, etc).[15]

Bazex's acrokeratosis is the most frequent paraneoplastic syndrome associated with cancer of the larynx and hypopharynx and is well documented in the literature.[3,5,15,19,22-28] Exceptionally, an association of different paraneoplastic cutaneous syndromes (Bazex's syndrome, hyperpigmentation, acquired ichthyosis, and pruritus) has been reported in a patient with laryngeal cancer.[22]

The syndrome often appears before the initial symptoms or diagnosis of the cancer. In a review of 93 patients with Bazex's syndrome, 63% revealed this syndrome an average of 11 months (range 1 to 72) before any clinical manifestation of the tumor.[5]

How this cutaneous syndrome develops in response to an underlying malignancy is unknown. Possible mechanisms include a cross-reactivity between skin antigens and the tumor, or the secretion of growth factors by the tumor.[5]

The differential diagnosis of these skin lesions includes psoriasis, dermatitis, pityriasis rubra pilaris, Reiter's syndrome, tinea, hereditary palmoplantar keratoderma, chronic arsenic ingestion, secondary syphilis, porphyria cutanea tarda, bullous pemphigoid, and acquired epidermolysis bullosa.[15,19]

In 91% (64/70) of the patients reported with this syndrome, the cutaneous lesions improved significantly after treatment of the underlying malignancy. In general, the cutaneous lesions are resistant to a variety of topical treatments.[5] The recognition of this cutaneous paraneoplastic syndrome may lead to the diagnosis and treatment of the underlying cancer.[21,29]

This condition responds to the removal of the primary cancer[20] and may respond to etretinate (Tigason),[30] systemic retinoids,[31] and corticosteroids.[12] A trial of octreotide, a long-acting synthetic somatostatin analog, would be reasonable as a palliative measure in inoperable cases.[20]

Bowen's disease appears clinically as an asymptomatic, persistent, progressive, nonelevated, often red, scaly, or crusted skin plaque caused by an intraepithelial neoplasm, and it has sometimes been reported to have an internal malignancy. It may occasionally be associated with laryngeal cancer.[32] The designation of Bowen's disease as a paraneoplastic cutaneous syndrome has been questioned, however.[32]

Bullous pemphigoid is a fairly common subepidermal blistering disease and has been reported in association with many tumors, including carcinoma of the larynx.[33] The association of bullous pemphigoid with malignancies still remains controversial, nonetheless.

Dermatomyositis is an inflammatory disease affecting the skin and skeletal muscle, causing widespread degenerative and inflammatory changes. The disease is characterized clinically by progressive proximal, symmetric muscle weakness and skin lesions. Its cause is unknown. Approximately 15% to 20% of cases are associated with underlying malignancies, most commonly carcinomas of the lung, breast, stomach, ovary, and kidney. In elderly patients in particular, it has also been reported in association with laryngeal carcinoma.[34,35]

Pruritus is rare in patients with a laryngeal malignancy. In a letter to the editor, Rantuccio[36] described 1 patient with "idiopathic" pruritus who subsequently developed cancer of the larynx. Paraneoplastic pruritus has been reported in patients with hematologic malignancies, particularly Hodgkin's lymphoma, and with solid tumors.[36-39] Only generalized pruritus (not localized pruritus) may be an uncommon symptom of malignancy.

Sweet's syndrome (acute febrile neutrophilic dermatosis) was originally described by Robert Douglas Sweet,[40] a British dermatologist, in 1964. It may occur as a cutaneous paraneoplastic syndrome. It has been associated mainly with hematologic disorders such as leukemia (acute myelogenous leukemia in particular) and with solid tumors.[41] The female sex is more often involved, and a recent upper respiratory tract infection and elevated erythrocyte sedimentation rate are reportedly frequent findings. The clinical characteristics included pyrexia, neutrophilia, anemia, painful erythematous cutaneous plaques, and nodules, mainly localized on the upper extremities, face, and neck. This condition has been reported in association with solid tumors of the breast and of the genitourinary and gastrointestinal organs. The absence of fever or neutrophilia does not rule out the possibility of Sweet's syndrome in patients with solid tumors.[42] Microscopically, the cutaneous lesions are characterized by a dense neutrophilic infiltrate primarily involving the superficial dermis. Exceptionally, Sweet's syndrome may occur in association with a squamous cell carcinoma of the pyriform sinus.[42] A complete malignancy workup is indicated in patients after the onset of Sweet's syndrome skin lesions, because an unsuspected primary or recurrent cancer may be detected. Approximately 10% to 20% of patients reported with Sweet's syndrome had an associated neoplasm.[42] Corticosteroid therapy is the treatment of choice. All the manifestations of Sweet's syndrome improved dramatically with corticosteroid therapy, regardless of the response of the associated neoplasm to tumor-directed therapy.[41,43] Potassium iodide and colchicine have also proved effective therapeutic alternatives.

Yellow nail syndrome has been reported in a patient with laryngeal carcinoma, and this disorder regressed after excision of the cancer.[44] The syndrome is characterized by a yellow or greenish discoloration of the nails preceded and accompanied by stunted growth and is associated with lymphedema (usually confined to the ankles but sometimes involving other areas) and occasionally also with bronchiectasis and sinusitis.

Tylosis (palmarum et plantarum) is characterized by hyperkeratosis of the palms and soles; it is often associated with esophageal cancer, and more rarely, with laryngeal cancer.[45] This hereditary disorder is assumed to be paraneoplastic.

PARANEOPLASTIC ENDOCRINE SYNDROMES

Paraneoplastic endocrine syndromes are caused by the production of polypeptide hormones and are usually associated with lung cancer, medullary carcinoma of the thyroid, breast cancer, carcinoids, etc. Endocrine syndromes are very rarely associated with malignant laryngeal neoplasms.

Carcinoid syndrome (carcinoidosis or argentaffinosis), as its name suggests, is most commonly associated with metastatic carcinoid tumors involving various organs (lung, stomach, colon, ovary, etc). This syndrome occurs very rarely in patients with small cell lung cancer. The 4 main clinical components of this syndrome are skin flushing, usually localized in the face and upper trunk, episodic watery diarrhea, manifestations of carcinoid heart disease, and wheezing. Less common changes include dermatitis and episodes of depression. Few patients dis-

TABLE 1. PARANEOPLASTIC SYNDROMES ASSOCIATED WITH LARYNGEAL NEUROENDOCRINE CARCINOMAS

Authors	*Year*	*Syndrome*	*No. of Cases*	*Type of Tumor*	*Follow-up*
Trotoux et al[50]	1979	SIADH	1	SCNC	DOD
Medina et al[52]	1984	Eaton-Lambert	1	SCNC	DOD
Bishop et al[51]	1985	ACTH	1	SCNC	DOD
Baugh et al[47]	1987	Carcinoid	1	AC	DOD
Takeuchi et al[53]	1989	SIADH	1	SCNC	DOD
Wenig and Gnepp[48]	1989	Carcinoid	1	TC	NED*
Myers and Kessimian[54]	1995	SIADH	1	SCNC	DOD
Overholt et al[49]	1995	Carcinoid	1	AC	DOD

SIADH — syndrome of inappropriate antidiuretic hormone, SCNC — small cell neuroendocrine carcinoma, DOD — dead of disease, ACTH — adrenocorticotropic hormone, AC — atypical carcinoid, TC — typical carcinoid, NED — no evidence of disease.

*Personal communication (1997).

play all the symptoms.

The majority of typical and atypical carcinoids of the larynx reported in the literature have been nonfunctional[46] and therefore did not lead to clinical syndromes such as carcinoid syndrome, adrenocorticotropic hormone (ACTH) syndrome, etc. Three cases of laryngeal carcinoid tumors (1 typical and 2 atypical) associated with a carcinoid syndrome have been reported, however.[47-49] One case, of typical carcinoid tumor, was identified from the files of the Department of Otolaryngic Pathology, Armed Forces Institute of Pathology, Washington, DC. Based on the diagnosis of a malignant tumor, a total laryngectomy was performed. The patient remained disease-free for 3 years, then noted a lump in his left neck that proved to be a metastasis from the primary laryngeal neoplasm. A left-sided radical dissection was performed, revealing multiple lymph node involvement. Six months later, the patient presented to his physician complaining of fever, malaise and right-sided upper quadrant abdominal pain. A computed tomography scan of the abdomen revealed multiple liver nodules that were submitted to biopsy and proved morphologically identical to the laryngeal neoplasm. This patient developed carcinoid syndrome following the liver metastases. Urinalysis revealed a 5-hydroxy indolacetic acid (5-HIAA) level increased to 195 mg/24 h (1 to 5 mg/24 h is normal), which decreased following therapy with streptozotocin and 5-fluorouracil. The patient was much improved after treatment and was alive and well at last follow-up[48] (also personal communication, 1997).

The second case was a supraglottic atypical carcinoid. Twenty-six months after therapy, the patient was admitted with abdominal cramps, distention, alternating diarrhea and constipation, and a generalized warm feeling. Urinary vanillylmandelic acid and 5-HIAA levels were normal. Multiple liver metastases and a retroperitoneal mass were found. The patient died 41 months after surgical treatment.[47]

The third case involved a 57-year-old man with an atypical carcinoid of the aryepiglottic fold. The patient presented with sore throat, flushing, diarrhea, and hypertension consistent with a carcinoid syndrome. The patient underwent supraglottic laryngectomy and modified radical neck dissection and remained well for 1 year. However, he developed recurrent local disease with evidence of distant metastases to the small bowel, mesentery, and skin. An attempt to control the disease with chemotherapy was unsuccessful, and the patient died after 26 months.[49]

Of the 8 patients reported with various paraneoplastic syndromes (carcinoid syndrome, Schwartz-Bartter syndrome, Eaton-Lambert syndrome, ACTH syndrome) in association with neuroendocrine carcinomas of the larynx (typical carcinoid, atypical carcinoid, small cell neuroendocrine carcinoma), 7 died (Table 1[47-54]).

Ectopic *ACTH syndrome* in association with a squamous cell carcinoma of the larynx was reported by Imura et al[55] in 1975. The authors described a Japanese patient, a 66-year-old man, who underwent surgical excision of the larynx for squamous cell carcinoma. One year later, he developed hyperadrenocorticism, and postmortem examination revealed metastases to the liver and other organs. Bioassayable and immunoassayable ACTH, as well as bioassayable melanocyte-stimulating hormone, were detected in the metastatic tumor tissue.[55]

In 1985, Bishop et al[51] reported the first case of laryngeal small cell neuroendocrine carcinoma associated with ectopic ACTH syndrome. The cell cytoplasm was immunoreactive for ACTH, gastrin-releasing polypeptide, neuron-specific enolase, β-endorphin, calcitonin, and keratin by indirect immunoperoxidase techniques.

Ectopic production of ACTH by tumors does not always result in classic Cushing's syndrome,[55] which was absent in the 2 mentioned reported cases.[51,55]

Schwartz-Bartter syndrome (syndrome of inappropriate secretion of antidiuretic hormone [SIADH]) was first described by Schwartz et al[56] in 1957. It is characterized by excessive blood levels or actions of vasopressin associated with hyponatremia without edema. The inability to excrete a dilute urine implies a subsequent retention of ingested fluids, with expansion of the extracellular fluid volume without edema. Hyponatremia is due both to sodium dilution in a larger extracellular fluid volume, and to its higher urinary excretion caused by its decreased reabsorption in the proximal renal tubular tract because of the increased extracellular fluid volume.

This condition is the most frequently detected paraneoplastic syndrome in patients with small cell lung cancer,[57] but it has been associated with many different types of tumors, including pancreatic carcinoma, duodenal carcinoma, prostatic carcinoma, bladder carcinoma, mesothelioma, lymphoma, Hodgkin's disease, acute myelogenous leukemia, thymoma, and adrenocortical carcinoma.

In the head and neck region, in particular, SIADH is a well-recognized form of paraneoplastic syndrome that may accompany malignancies. In a recent review of the literature, Ferlito et al[58] collected 70 cases of this syndrome associated with cancers of the head and neck. The most common site of occurrence was the oral cavity, with 29 cases detected; the larynx was involved in 13 patients, and the hypopharynx in 7. The most frequent histologic tumor type was squamous cell carcinoma,[59-63] and 3 cases were small cell neuroendocrine carcinoma.[50,53,54] The SIADH may precede the presentation of the cancer by several weeks or months.

The first 2 cases of this syndrome associated with squamous cell carcinoma of the larynx were described in 1976, by Moses et al.[59] Other cases associated with squamous cell carcinoma were subsequently reported by various authors.[60-63]

In 1979, Trotoux et al[50] described the first case of small cell neuroendocrine carcinoma of the subglottic region associated with SIADH. The patient presented with initial headache, confusion and temporospatial disorientation, hyperreflexia, hyponatremia, hypochloremia, serum hypo-osmolarity, reduced hematocrit, negative free water clearance, and high plasma levels of antidiuretic hormone. The diagnosis of the subglottic tumor was only reached 3 months later.

In 1989, Takeuchi et al[53] reported a case of small cell neuroendocrine carcinoma of the larynx in a 53-year-old man. The tumor was associated with SIADH, and hyponatremia persisted until the patient's death, despite the administration of salt. Postmortem examination revealed no central nervous system lesions or lung diseases.

In 1995, Myers and Kessimian[54] described a patient with small cell neuroendocrine carcinoma of the larynx who had clinical complications of SIADH. The diagnosis of this endocrine syndrome was confirmed by the finding of serum hyponatremia and hypo-osmolarity, urine hypo-osmolarity, and an increased urinary sodium concentration. Careful patient evaluation identified only the laryngeal neoplasm as the cause of SIADH and excluded any other potential causes (pulmonary neoplastic or non-neoplastic diseases, central nervous system lesions, drugs).

Hypercalcemia is the most common metabolic complication of malignancy[64] and often presents in the terminal stage of neoplastic disease. It has been associated with many neoplasms, including carcinomas (especially of the head and neck, esophagus, lung, kidney, and ovary), sarcomas, and hematopoietic malignancies.[65,66] Bone metastasis is an uncommon cause.[67] Many cancers produce hypercalcemia by an ill-understood hormonal mechanism. Nonmetastatic hypercalcemia is not uncommon in patients with laryngeal malignancies.[67] Clinical signs of mild hypercalcemia include dehydration, anorexia, nausea, vomiting, constipation, ileus, fatigue, and confusion. Severe hypercalcemia is considered a medical emergency.[68] Hypercalcemia complicating malignant disease is a poor prognostic sign and correlates with advanced disease, but it is important to distinguish this from hypercalcemia due to other causes, ie, sarcoidosis, primary hyperparathyroidism, or thiazide therapy. Treatment of malignancy-induced hypercalcemia is primarily directed toward controlling the underlying cancer.[68]

PARANEOPLASTIC HEMATOLOGIC SYNDROMES

Paraneoplastic hematologic syndromes may occur in many neoplasms, but have only occasionally been observed in association with laryngeal cancer.

Trousseau's syndrome (thrombophlebitis migrans, migratory thrombophlebitis, disseminated intravascular coagulation, or thromboembolism) was first reported, in 1865, by Armand Trousseau,[69] who described a high incidence of venous thrombosis in a series of patients with gastric carcinoma. Historically, Trousseau's syndrome has been associated with pancreatic carcinoma, but it has also been reported in association with stomach, breast, lung, colon, and ovarian carcinoma.[70] The prevalence of this syndrome with head and neck cancers is less than 1%, and it has only occasionally been observed in laryngeal carcinoma.[71] This process is characterized by disseminated intravascular coagulation and is detected by laboratory tests (elevated levels of fibrin, thrombocytosis, hyperfibrinogenemia). An unexplained thromboembolism may indicate the presence of a malignancy before any signs or symptoms of the tumor itself become apparent.[72]

PARANEOPLASTIC NEUROLOGIC SYNDROMES

Paraneoplastic neurologic syndromes occur frequently in cancer patients, but have been described

TABLE 2. PARANEOPLASTIC SYNDROMES OCCURRING IN PATIENTS WITH LARYNGEAL AND HYPOPHARYNGEAL CANCERS

Syndrome	*Authors*	*Type of Tumor*
Cutaneous syndromes		
Acanthosis nigricans*	Miller and Davis[1]	SCC
	Oppolzer et al[2]	
Bazex's syndrome*	Bazex et al[3]	SCC
	Bolognia et al[5]	SCC
	Laccourreye et al[15]	SCC
	Mounsey and Brown[19]	SCC
	Bazex et al[22]	SCC
	Sarkar et al[23]	SCC
	Miquel et al[24]	SCC
	Legros et al[25]	SCC
	Gaillard et al[26]	SCC
	Blanchet et al[27]	SCC
	Colomb et al[28]	SCC
Bowen's disease	Cohen[32]	SCC
Bullous pemphigoid	Hodge et al[33]	SCC
Dermatomyositis*	Bonnetblanc et al[35]	SCC
Pruritus*	Rantuccio[36]	SCC
Sweet's syndrome*	Cohen et al[42]	SCC
Yellow nail syndrome	Guin and Elleman[44]	SCC
Tylosis	Haines[45]	SCC
Endocrine syndromes		
Carcinoid syndrome*	Baugh et al[47]	AC
	Wenig and Gnepp[48]	TC
	Overholt et al[49]	
ACTH syndrome*	Bishop et al[51]	SCNC
	Imura et al[55]	SCC
Schwartz-Bartter syndrome*	Trotoux et al[50]	SCNC
	Takeuchi et al[53]	SCNC
	Myers and Kessimian[54]	SCNC
	Moses et al[59]	SCC
	Zohar et al[60]	SCC
	Talmi et al[61]	SCC
	Roth et al[62]	SCC
	Kandylis et al[63]	SCC
Hypercalcemia*	Angel et al[67]	SCC
Hematologic syndromes		
Trousseau's syndrome*	Nikšic and Balogh[71]	SCC
Neurologic syndromes		
Cerebellar degeneration*	Garcin and Lapresle[73]	SCC
	Müller et al[74]	SCC
Eaton-Lambert myasthenic syndrome*	Medina et al[52]	SCNC
	Fontanel et al[80]	SCC

SCC — squamous cell carcinoma, AC — atypical carcinoid, TC — typical carcinoid, ACTH — adrenocorticotropic hormone, SCNC — squamous cell neuroendocrine carcinoma.

*True paraneoplastic syndrome.

only occasionally in association with laryngeal cancers.

Cerebellar degeneration, or cerebellar cortex degeneration, is a relatively common paraneoplastic effect of small cell lung cancer, but it also occurs with other types of lung cancer, as well as with tumors arising elsewhere (ovary and breast cancer). It has also been occasionally reported in association with laryngeal cancer.[73,74] It is characterized by subacute symptoms of diffuse cerebellar dysfunction: vertigo, dysarthria, intention tremor, and progressive limb and truncal ataxia. Ocular disturbances, nystagmus, ocular dysmetria, and opsoclonus are also common. This syndrome may be caused by immunologic cross-reactions, and antineural antibodies are found in about half the patients. The most specific autoantibody is the anti–Purkinje cell antibody, found in women with gynecologic tumors.[75] Paraneoplastic syndromes usually occur before the malignancy is diagnosed, and the best described are those associated with specific antibodies.[76]

Eaton-Lambert myasthenic syndrome was reported in abstract form by Lambert et al[77] in 1956 and again in more detail by Eaton and Lambert in 1957,[78] though the same condition had been reported by Gray and Halton[79] in 1948. It is usually seen in association with lung cancer, but may also be associated with laryngeal cancer. Fontanel et al[80] described a case of this syndrome in a 58-year-old man that led to the detection of a squamous cell carcinoma of the larynx.

In 1984, Medina et al[52] reported a case of primary small cell neuroendocrine carcinoma of the larynx associated with clinical and electromyographic evidence of the myasthenic syndrome. The upper extremities may become involved. Ocular and bulbar involvement is rare and, when present, less severe than in the case of myasthenia gravis. Deep tendon reflexes are hypoactive or absent, but sensory function is preserved. Unlike myasthenia gravis, muscle strength improves with exercise. Electromyography is essential in the diagnosis of this syndrome, and electromyographic findings differ from those observed in patients with myasthenia gravis. The electrophysiological diagnosis of Eaton-Lambert syndrome relies on the observation of a progressive increase in the amplitude — by 200% or more — of the compound muscle action potential evoked by repetitive supramaximal nerve stimulation.

Diagnostically useful serum and cerebrospinal fluid autoantibodies have recently been identified in several neurologic paraneoplastic syndromes, including cerebellar degeneration and Eaton-Lambert myasthenic syndrome.

Table 2[1-3,5,15,19,22-28,32,33,35,36,42,44,45,47-55,59-63,67,71,73,74,80] summarizes the paraneoplastic syndromes reportedly occurring in patients with cancer of the larynx and hypopharynx.

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BOOK REVIEWS

Diseases and Disorders of Ear, Nose, and Throat

C. A. J. Prescott. Soft cover, illustrated, indexed, 215 pages, 1998. Oxford, Cape Town, South Africa, $29.95.

Purpose: 1) To refresh knowledge of essential ENT anatomy and physiologies so that "normal" can be recognized. 2) To teach an organization structure to the examination of the ear, nose, throat, head, and neck region to present students with essential core knowledge of ENT disorders. 3) To present a practical approach to ENT disorders. 4) To establish guidelines for specialist referrals in ENT disorders. 5) To assess the competence of students regarding their core knowledge, examination skills, and ability to diagnose and manage common ENT disorders. 6) To stimulate in some students the desire to further pursue ENT through elective research projects, further training, or specialist training.

Contents: It is divided into 3 sections to include the 1) ear, 2) nose and sinuses, and 3) throat, head, and neck.

Attributes: The book is very comprehensive for its size, in that the author gives clear explanations of specialized concepts and a "core" knowledge of ENT disorders commonly presenting in the primary care setting.

Limitations: Certain pathological illustrations necessitate color, such as acute otitis media or a middle ear cholesteatoma, and this is lacking in the book.

Applicability: This book is directed to the medical student and health care professional.

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Essential Otolaryngology–Head and Neck Surgery

K. J. Lee, editor. Soft cover, illustrated, indexed, 1,173 pages, 1999. Appleton & Lange, Stamford, Conn, $75.

Purpose: To serve as a guide for broad preparation, as well as a practical and concise reference text reflecting contemporary concepts in clinical otolaryngology.

Contents: Forty-one chapters, covering all aspects of otolaryngology and selected complementary topics, such as anesthesia, head and neck radiology, and pharmacology. Numerous tables and diagrams. Each chapter contains references and a bibliography.

Attributes: A relatively concise review of the specialty in an easy-to-read format. Each chapter provides the reader with a quick review of the subject, along with important aspects to focus on. The text ends with a "Highlights and Pearls" chapter containing over 150 pages of high-yield material commonly found on board examinations.

Limitations: The chapters are not sequenced in an organized fashion. For example, the chemotherapy chapter is followed by nasal endoscopy and then antimicrobial therapy; chapters on head and neck cancer, along with the nose and sinus chapter, are later in the text. Further, chapters from the earlier editions remain in outline format, with newer chapters written in a text format. Certain important topics were also omitted, such as congenital hearing loss caused by cytomegalovirus and repositioning maneuvers for benign paroxysmal positional vertigo.

Applicability: Since 1973, innumerable otolaryngology residents have used this text to prepare for their board examinations. It is also a valuable reference for the practicing otolaryngologist who wants to quickly review a subject.

MARK D. GIBBONS, MD
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Office-Based Surgery in Otolaryngology

John H. Krouse, Joseph P. Mirante, and Dewey A. Christmas, Jr. Hard cover, illustrated, indexed, 273 pages, 1999. Saunders, Philadelphia, Pa, $75.

Purpose: To describe a broad range of surgical procedures that could be performed in the average otolaryngologist's office. The intensity of surgery has been classified (elsewhere) as level 1, involving only local anesthetic agents, level 2, which makes use of preoperative medications and perioperative intravenous sedation, and level 3, involving the use of general anesthetics. In this book, the majority of procedures described are performed in level 1, in an attempt to expand the list of procedures that will be practical with local anesthesia alone.

Contents: Plastic surgery and sleep disorder surgery make up the greater number of the chapters, which number 23 in all. Individual chapters involve each aspect of the head and neck specialty. Equipment, philosophy, emergency, and coding issues make up the rest of the text.

Attributes and Limitations: The book is momentous from the standpoint that office-based surgery is an emerging trend. Have the authors extended level 1 surgery to make is worthwhile for the reader? Typically, procedures that the authors are now including in level 1 would include sinus surgery, turbinectomy, septoplasty, serial tonsillectomy, and maybe some aspects of plastic surgery. Most all other procedures mentioned by the authors are done routinely by practicing otolaryngologists and are routine practice. I understand the authors are experts in their field, and indeed, all chapters are well done and beneficial to the reader.

Applicability: To those interested in extending their practice into office-based surgery.

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Ann Otol Rhinol Laryngol 109:2000

CRANIOFACIAL BALLPOINT PEN INJURY: ENDOSCOPIC MANAGEMENT

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Penetrating facial injuries are not infrequent. There have been isolated case reports of unusual penetrating craniofacial trauma. We describe an unusual case of a 22-month-old child who suffered an external orbital injury from a ballpoint pen that penetrated the orbit, lamina papyracea, posterior ethmoid sinuses, and sphenoid sinus. Endoscopic sinus surgery was performed to extract the ballpoint pen nib after localization with computed tomography. Careful pediatric endoscopic sinus surgery techniques permitted safe foreign body extraction with minimal morbidity.

KEY WORDS — endoscopic sinus surgery, foreign body, paranasal sinus.

INTRODUCTION

Penetrating facial injuries are not infrequent. Low-velocity gunshot and stab wounds account for the majority of such injuries. The maxillary and ethmoidal sinuses and orbit are particularly at risk.[1] The frontal and sphenoid sinuses are involved occasionally with gunshot wounds to the face. There have been isolated case reports of unusual penetrating craniofacial trauma. Air gun pellets have been removed from the maxillary and sphenoid sinuses.[2] Craniofa-

[redacted]

ecchymosis and edema. Radiographs obtained in the ED could not rule out an upper airway foreign body (Fig 2). The patient was taken urgently to the operating room for foreign body removal and completion of the ophthalmologic evaluation. No significant orbital damage was noted. Due to the angle of penetration, the nib was thought to rest within the nasal cavity or nasopharynx. The nib could not be identified through the entrance wound or by nasal endoscopy. A subsequent CT scan confirmed that the nib was

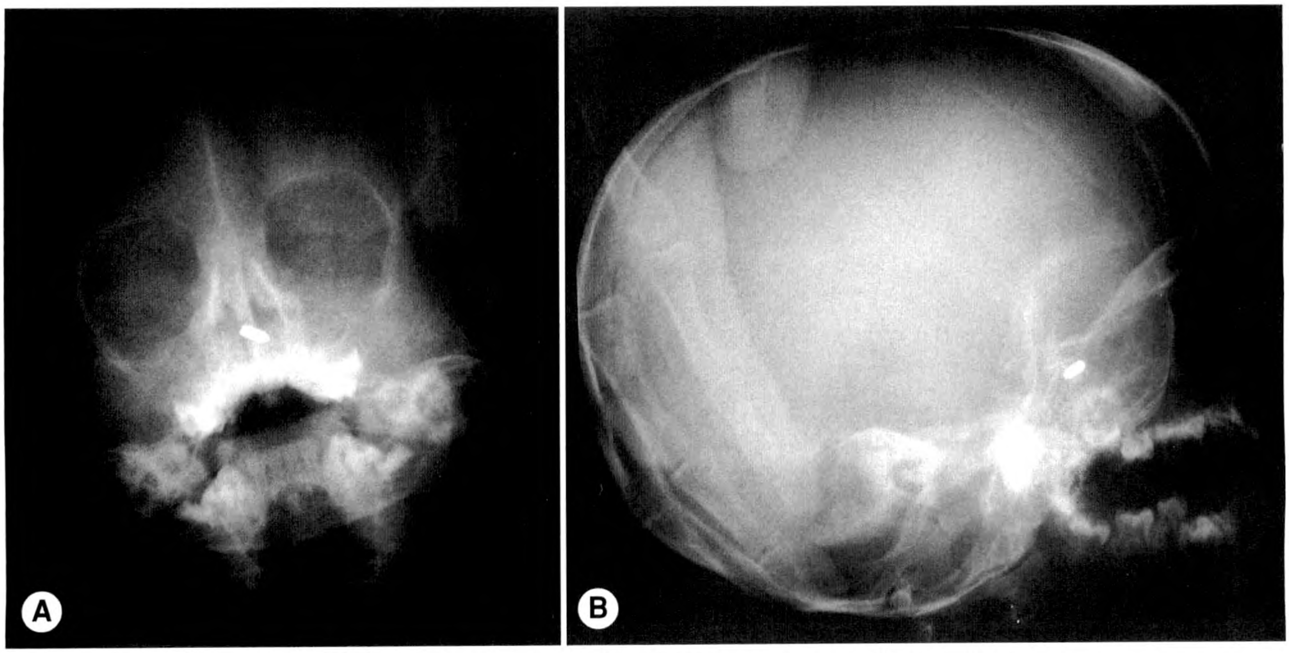


Fig 2. Radiographs showing pen nib. A) Anteroposterior view. B) Lateral view.

was given intravenous antibiotics and underwent endoscopic sinus surgery to include a right uncinectomy, anterior and posterior ethmoidectomies, and a sphenoidotomy. The nasal mucosa was edematous, and purulence filled the ethmoid air cells. When the pneumatized sphenoid was opened, the nib was palpated in an admixture of black ink and pus (Fig 4A,B). The nib was removed and the surgically opened sinuses were irrigated (Fig 4C,D). The posterior lamina papyracea and periorbita were violated by the pen injury, and a small amount of periorbital fat was identified near the orbital apex and left undisturbed within the lateral posterior ethmoid air cell. No cerebrospinal fluid leak was noted, and the blood loss was less than 10 mL. The child made an uneventful recovery and suffered no adverse sequelae.

DISCUSSION

This case illustrates a sphenoidal foreign body resulting from a fall upon a seemingly innocuous ballpoint pen. The potential for devastating craniofacial injury when this occurs is clear. In our case, the patient was spared serious orbital, carotid artery, optic

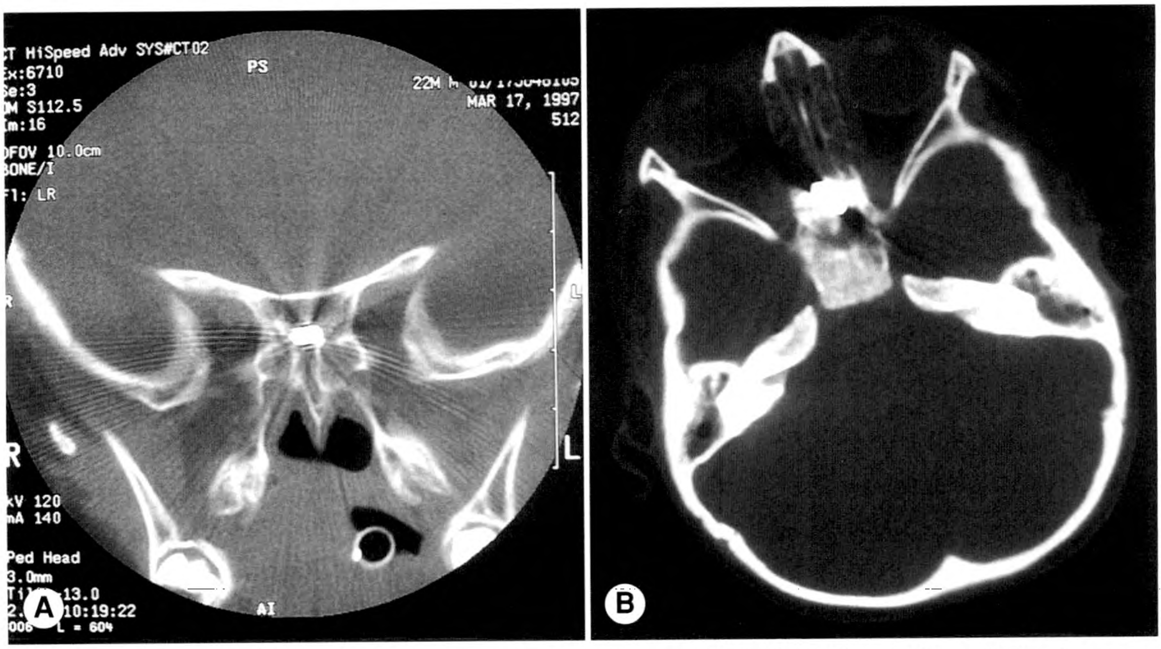
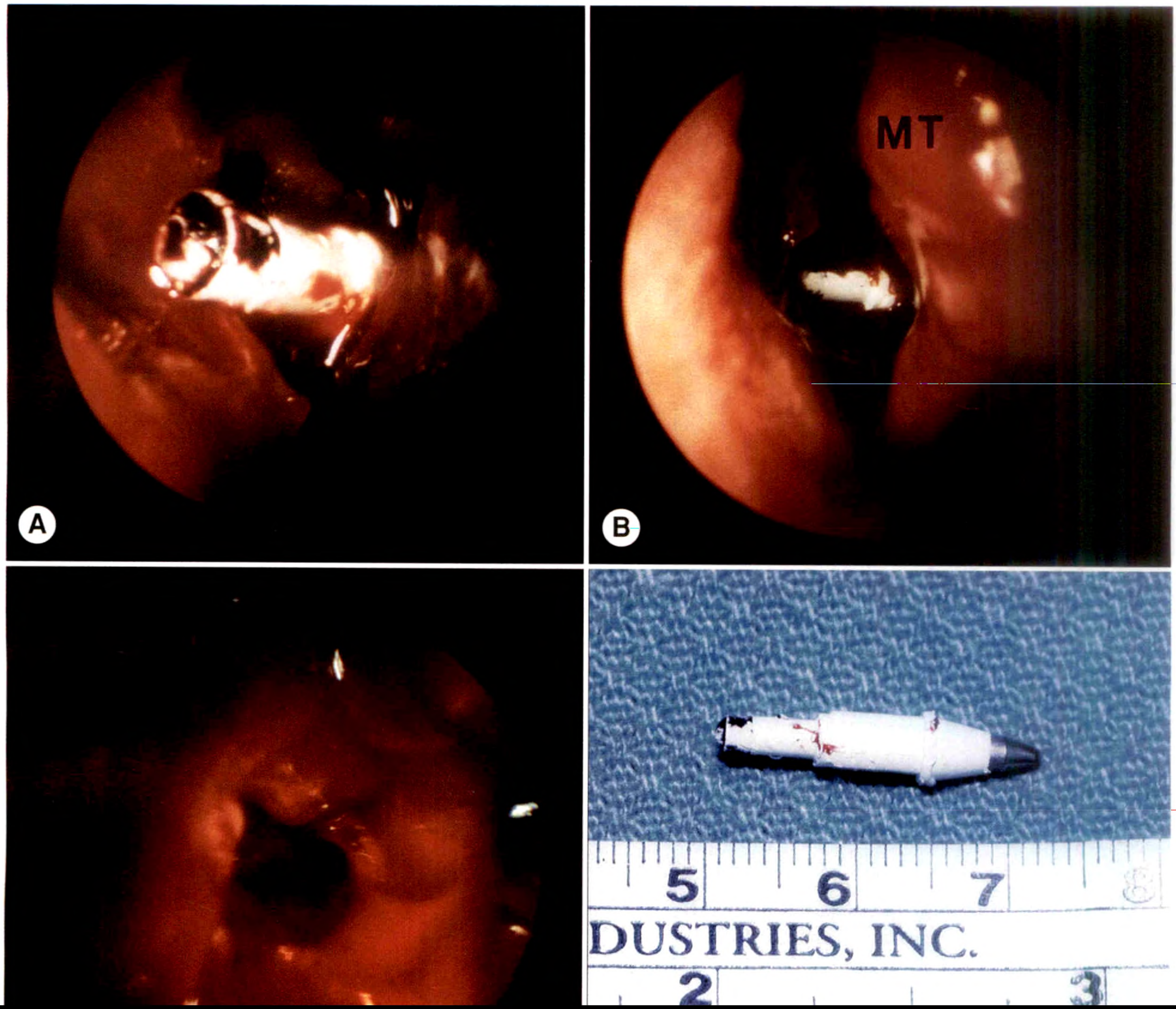


Fig 3. Computed tomography. A) Coronal view, demonstrating pen nib in sphenoid at skull base. B) Axial view.



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Ann Otol Rhinol Laryngol 109:2000

PRELIMINARY CLINICAL RESULTS OF WINDOW PARTIAL LARYNGECTOMY: A COMBINED ENDOSCOPIC AND OPEN TECHNIQUE

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Endoscopic laser resection for anterior commissure glottic carcinoma is difficult, because of inadequate exposure and close proximity to the underlying cartilage. A technique combining endoscopic carbon dioxide laser incision and an external approach creating a window in the thyroid cartilage was initially tested in a canine study and then performed in 5 patients. All patients were men, with T1 or T2 glottic or supraglottic cancer involving the anterior commissure, and had failed radiation treatment. The true or false vocal fold tumors were excised along with the paraglottic space and adjacent cartilage, with preservation of the remaining thyroid framework. The reconstruction was accomplished with placement of a sternohyoid muscle flap, by use of either a bipedicled muscle flap with overlying skin or a unipedicled muscle flap with a graft of free mucosa. The graft was secured in place with fibrin glue and laser soldering. Follow-up ranged from 11 months to 4 years and included biopsies. All patients had voice recordings before and after surgery. A tracheostomy was avoided in all patients. The hospital stays were 4 to 13 days. The voice quality was good after surgery. One patient died of unrelated causes 18 months after his surgery without evidence of recurrence. The other patients are still alive with no evidence of disease. The only complication was subcutaneous neck emphysema in 1 patient that spontaneously resolved. The results showed a satisfactory anatomic reconstruction and acceptable functions. We believe that this new combined technique is oncologically sound, may overcome the limited access seen with the endoscopic technique and the excessive cartilage resection seen with external partial laryngectomy, avoids a tracheostomy, and shortens hospital stays.

KEY WORDS — endoscopic laser resection, glottic carcinoma, window partial laryngectomy.

INTRODUCTION

The primary goal of any oncological surgery of the larynx is to obtain good control of local disease. Partial laryngeal surgery has 2 essential objectives: to conserve the physiological functions of the larynx, and to completely remove the tumor while achieving the same cure rate as a nonconservative operation.

These objectives are sometimes difficult to achieve with endoscopic microlaryngeal surgery, while the traditional partial laryngectomy may be too aggressive for a small laryngeal primary tumor. Endoscopic resection can also be suboptimal, because the margins may be close and difficult to evaluate properly. Several laryngeal conservation surgeries have been described: the laryngofissure for cordectomy, the frontolateral laryngectomy, the supraglottic laryngectomy, the near-total laryngectomy, and the supracricoid laryngectomy that was described by Piquet et al[1,2] and later by Laccourreye et al.[3] In some instances, however, these procedures can lead to the resection of more tissue than is needed for proper oncological control, thus compromising good functional results.

In an attempt to preserve the voice, indications for endoscopic procedures have been extended to T3 glottic and supraglottic tumors. In our experience, endoscopic laser excision has several limitations. It is usually difficult to extend the resection laterally into the paraglottic space and the area of the anterior commissure, due to the inability of angulating the laser beam or the axis of the microscope.

A new technique, termed window partial laryngectomy (WPL), was tested in a live canine model prior to clinical application and previously described in a published article.[4] It successfully combines the advantages of precise endoscopic laser incisions and external en bloc resection. In the canine study, WPL was found to be a useful addition to the existing conservation laryngectomy techniques, providing com-

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Beach, Florida, May 11-12, 1998.

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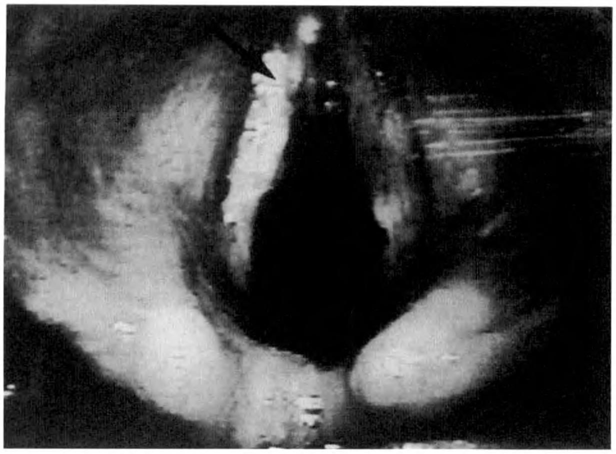

Fig 1. Endoscopic view showing left true vocal fold lesion involving anterior commissure (arrow).

plete resection of tumor growth and preservation of good laryngeal function.[4,5]

In this article, we review the technique and results in 5 patients who underwent a WPL for laryngeal neoplasm.

PATIENTS

This technique was performed in 5 patients with glottic or supraglottic carcinoma (Fig 1). Table 1 summarizes the patients' characteristics. Their ages ranged from 65 to 75 years. All patients were male.

In 4 patients, the anterior commissure was involved, and 1 patient had a right false vocal fold tumor. In 1 patient with a glottic lesion, the undersurface of the true vocal fold was involved and was felt not to be resectable endoscopically. Four patients had radiotherapy 9 to 24 months prior to the recurrence. The patient with a T1 supraglottic tumor had irradiation for a glottic tumor 18 years prior to his second primary.

Preoperative evaluation included an initial office examination, computed tomography of the larynx and neck, a diagnostic laryngoscopy and biopsy, and an evaluation by a speech therapist. In addition, all patients had videostroboscopy before and after the surgery, as well as speech therapy after the surgery.

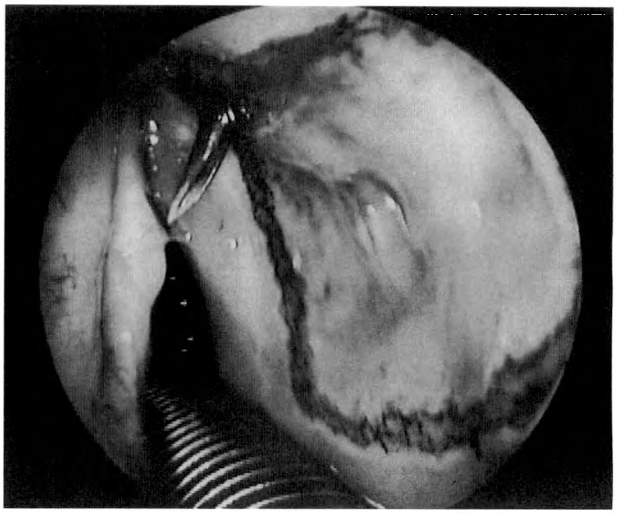

Fig 2. Endoscopic view showing right false vocal fold tumor that has been outlined with carbon dioxide laser. Twenty-gauge needle was inserted through thyroid cartilage, into endolarynx at level of ventricle, to help guide in making external incisions.

TECHNIQUE

The operation is performed under general anesthesia and orotracheal intubation with a laser-safe tube. With a microspot carbon dioxide laser (250 μm) at power settings of 1.5 to 2.5 W, incisions are made to outline the lesion. The laryngeal framework is then exposed through a collar neck incision. The sternohyoid muscle is retracted and a thyroid cartilage window is made at the level of the true vocal fold. Under endoscopic guidance, the incisions are extended deep into the laryngeal mucosa (Fig 2), thus connecting the external incision with true vocal fold incisions made endoscopically. A complete en bloc resection is then made, including the true vocal fold mu-

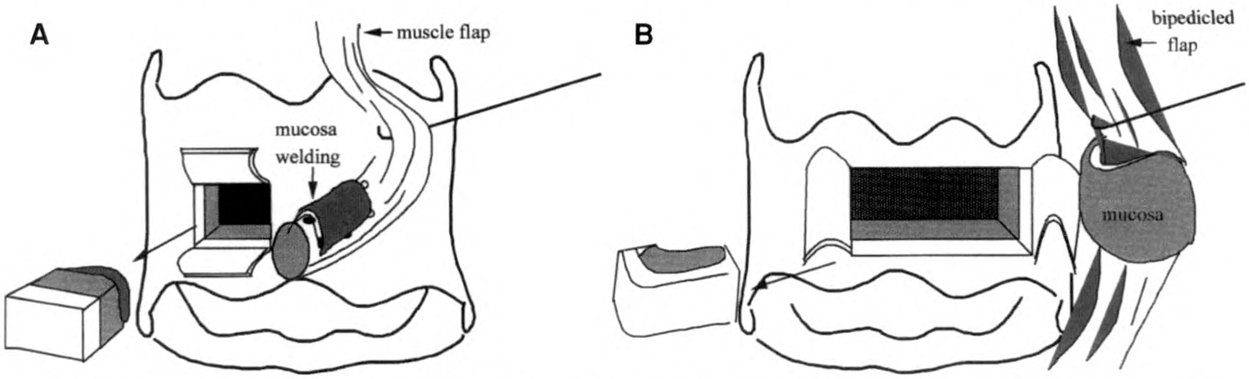

Fig 3. Schematic drawing of operation. A) Pseudocord reconstructed with muscle flap and mucosal graft welding technique. B) Larger glottic defect with anterior commissure repaired with bipedicled rotary muscle flap and overlying mucosa.

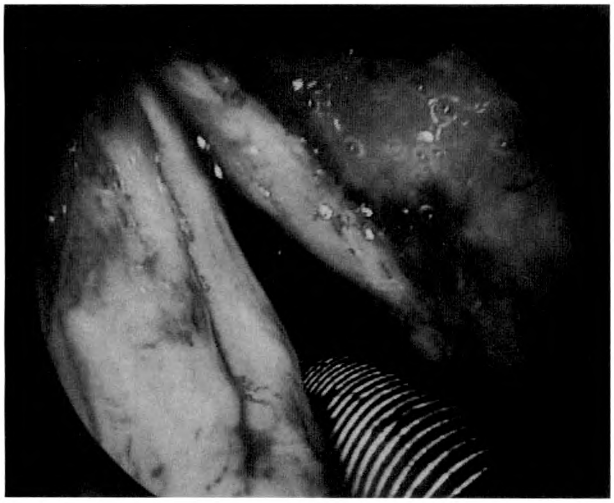

Fig 4. Endoscopic view of defect covered by fibrin glue.

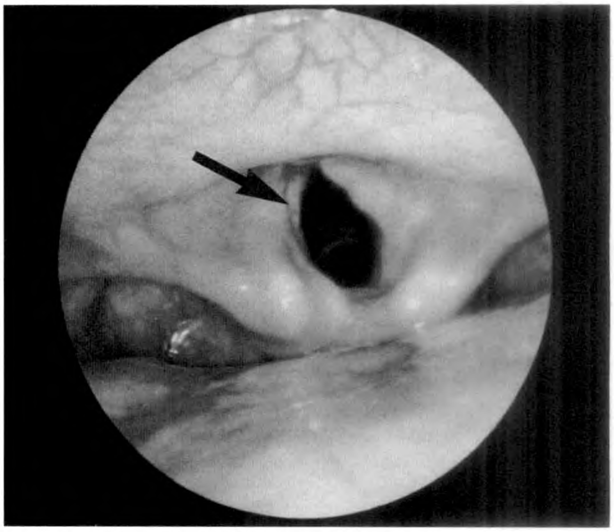

Fig 5. Reconstructed left side of glottis (arrow) 3 months after surgery.

cosa and muscle, the paraglottic space tissue, the inner perichondrium, and a piece of the thyroid cartilage (Fig 3). It is of utmost importance to mark the specimen and orient it with the pathologist. The resection is made under frozen section control.

A sternohyoid muscle flap is prepared, and a piece of buccal mucosal graft that has been previously harvested is soldered onto the muscle flap with a mixture of 1% indocyanine green dye and human fibrin adhesive with an 810-mm diode laser (Oculight, Iris Medical, Mountain View, Calif; Fig 4). The diode laser setting is 400 mW at 1-second exposures, and the laser beam is used to spot-solder around the edges of the graft in order to make it watertight and airtight. The pedicled muscle is inserted into the laryngeal window to serve as a new true vocal fold, with the mucosa facing the endolarynx, and good positioning is ensured endoscopically. Closure of the window is accomplished by careful reapproximation of the external thyroid perichondrium and skin. Any remaining defect between the graft and the adjacent laryngeal mucosa is sealed by the endoscopic solder technique previously described in the canine study.[4] A Penrose drain is inserted, and a pressure dressing applied. The patients are extubated immediately after the procedure and a tracheostomy is avoided.

Steroids and antibiotics are administered for 24 to 48 hours. Clear liquids are started the next morning if no emphysema is noted. The patient is advised voice rest for 1 week.

RESULTS

The results are summarized in Table 1. The follow-up ranged from 11 to 58 months. Four patients were closely followed up from 18 to 58 months with no evidence of recurrence. Two patients exhibited changes in the operative site and were submitted again to biopsy 9 months and 15 months after surgery, and were found to be free of disease. One patient died 18 months after the operation due to a cardiac event, free of disease.

The resection margins were clear in all patients. All patients were successfully extubated at the end of the procedure, and none required a tracheostomy. The patient with a supraglottic cancer (case 5, false vocal fold) underwent a modified neck dissection

TABLE 1. PATIENT CHARACTERISTICS

Case	*Age*	*Sex*	*Site*	*Previous Treatment*	*Complications*	*Hospital Stay (d)*	*Time to Oral Intake (d)*	*Follow-up (mo)*	*Status*
1	70	M	True vocal fold, AC	XRT	Subcutaneous emphysema	13	7	58	NED
2	65	M	True vocal fold, AC	XRT	None	9	2	18 (died of MI)	NED
3	75	M	True vocal fold, AC	XRT	None	4	7	40	NED
4	70	M	True vocal fold, AC	XRT	Chest pain, MI ruled out	12	4	34	NED
5	68	M	False vocal fold	XRT*	None	4	2	11	NED

AC — anterior commissure, XRT — radiotherapy, NED — no evidence of disease, MI — myocardial infarction.

*Irradiation 18 years earlier for T1 glottic cancer.

TABLE 2. INDICATIONS FOR AND CONTRAINDICATIONS TO WINDOW PARTIAL LARYNGECTOMY

Indications	*Contraindications*
T1 or T2 laryngeal lesion	T3, T4 laryngeal lesion
Accessible to endoscopic visualization	Poor laryngeal exposure
Too extensive for endoscopic excision	
Anterior commissure involvement	

(levels 2, 3, and 4) and had no neck metastasis.

Patients were admitted to the intensive care unit for overnight observation. Perioperative steroids and antibiotics were administered routinely. Oral feeding was started the next day in 2 patients. One patient developed subcutaneous emphysema that delayed his feeding to 7 days, and 1 patient had persistent pain upon swallowing and was kept on nasogastric feeding for 7 days. His pain disappeared on day 7, and he was able to tolerate oral feeding. The reason for the prolonged discomfort was not clear.

The hospital stays for the patients were 13, 9, 4, 12, and 4 days. The 2 patients with the longest hospital stays were the ones who developed complications (subcutaneous emphysema and chest pain).

Follow-up revealed excellent healing of the defect and the anterior commissure, with a good voice in all the patients (Fig 5). A postoperative speech evaluation was performed by a speech pathologist, followed by several sessions of speech therapy. As expected, the patient with the false vocal fold cancer (case 5) had the best voice result after surgery, and his videostroboscopy findings returned to normal, with a good mucosal wave, 6 weeks after surgery. All other patients had persistent submucosal scarring and poor wave motion of the neocord mucosal graft. All patients, however, described their voices as very functional and were satisfied with the results.

In this small series, surgical complications were limited to subcutaneous emphysema in 1 patient, and chest pain in 1, both of which prolonged the hospital stay. The patient with subcutaneous emphysema was the first patient treated in the series. After this complication, the closure of the defect was improved by injecting more fibrin glue endoscopically and adding more bulk during the external closure.

Myocardial infarction was ruled out in the patient who developed chest pain.

DISCUSSION

Complete tumor resection is essential in oncological surgery, and therefore, the resection of a large T1 or T2 vocal cord carcinoma should ideally include underlying deeper tissue, even perichondrium, as well as cartilage, particularly if the tumor is at the anterior commissure. This is not possible endoscopically, because of limited exposure and access. In our cases, the WPL technique enabled the surgeon, after endoscopic laser delineation of margins, to use an external approach for adequate and relatively easy resection of the deeper tissue adjacent to the involved vocal fold, while preserving the framework of the thyroid cartilage. Currently, although the results are encouraging, the number of patients is small, and satisfactory long-term follow-up is lacking. Any questions about the long-term survival rate cannot, therefore, be answered.

TABLE 3. SELECTION CRITERIA

No cartilage invasion
Vocal process not involved
No extralaryngeal cancer
No subglottic cancer
Large T1, anterior commissure, T2
Mobile vocal fold

In vertical partial laryngectomy, tracheostomy is a standard practice to ensure adequate respiration until the laryngeal edema subsides. A tracheostomy tube is kept in place until an adequate airway returns, usually between 1 and 2 weeks after the operation. Postoperative care for the tracheostomy can be troublesome, with prolonged hospitalization and significant morbidity. There is also the added risk of infection or tracheal stenosis. With the use of this new approach, a tracheostomy is avoided, due to the minimal disturbance to normal airway structures and the laryngeal framework, in addition to the precise adjustment of the rima glottidis obtained by positioning the pseudocord under endoscopic guidance. Intraoperative and postoperative steroid administration is of great help in minimizing postoperative edema.

Aspiration is another major complication following standard partial laryngectomy. Its incidence varies with the extent of resection and the success of reconstruction, but as many as 20% of patients have some degree of aspiration. This symptom is thought to involve the dysfunction of 3 sphincters — the epiglottis, the aryepiglottic folds, and the true vocal folds — but most authors agree that true vocal fold closure plays a major role in preventing aspiration.[6,7] Resection of the arytenoid without reconstruction is also a major risk factor for aspiration. In our cases, a precise reconstruction of the true vocal fold defect was completed through the window approach, and the resections were carried through the vocal processes, leaving the body of the arytenoid in place. Additionally, a better swallowing function could be experienced, because most of the rigid laryngeal framework was preserved.

Eliachar et al[8] described a rotary door flap technique using a well-vascularized bipedicled sternohyoid muscle for laryngeal reconstruction. Overlying skin attached to the muscle is preserved as a reliable lining, and when the flap is rotated 180° on its longitudinal axis, a spiral tension is created.[8] In our animal study, the tension was important both in forming a new anterior commissure and in preventing soft tissue collapse after such a large resection.[5] In case 2, whose true vocal fold tumor involved the anterior commissure, the thyroid window had to be enlarged to ensure a complete resection of the entire left true vocal fold, the anterior commissure, and one third of the anterior right true vocal fold. In such a case with a large defect, a rotary door flap offers a satisfactory effect for reconstruction. The muscle gives structural support for the anterolateral walls of the larynx, while the skin graft cover can be mobilized, tailored, and folded to fill a large intralaryngeal defect, thereby reconstructing the entire true vocal fold bilaterally, as well as the anterior commissure.

In review of our experimental and clinical cases, there are 2 points deserving further attention. The first is to provide more tissue bulk for reconstruction of the true vocal fold, especially in using unipedicled muscle as a rebuilding material. In an experimental study reported, there was about a 23% reduction in the size of the flap after reconstruction of a pseudocord with an unipedicled sternohyoid flap.[9] For this reason, consideration should be given to the need for overcorrecting the defect. In addition, careful preparation of the pedicled muscle flap to preserve the blood supply and neural integrity reduces the chance of atrophy. In case of atrophy or retraction of the tissue, adding bulk to the pseudocord could theoretically be achieved via a later external thyroplasty or an endoscopic fat injection. Second, it is important to secure complete epithelium coverage to avoid granulation tissue and subsequent scarring. This is more important in the reconstruction of a large laryngeal defect. An adequate internal and external closure of the wound with assistance of the laser soldering technique helps reduce the possibility of postoperative infection and emphysema.

Our limited experience with the window approach has indicated some treatment success in 5 selected patients with early glottic and supraglottic cancer. The preservation of the laryngeal framework is the key to successful functional reconstruction of the glottis. The simplicity of this window approach, combining the advantages of endoscopic precision and orientation and an external approach for complete cancer resection, seems to meet the modern objectives of surgery in being less invasive and more cost-effective. The fact that only 5 patients have undergone this procedure in 4 years attests to the fact that careful patient selection, with strict selection criteria, is the key to success (Tables 2 and 3). Five other patients scheduled for this procedure were found to have more extensive disease at the time of surgery, and the procedures were converted to standard hemilaryngectomy or total laryngectomy.

So far, we have not treated patients with T3 tumors or extensive T2 tumors with marked subglottic or supraglottic involvement. Theoretically, WPL could be extended to include treatment of patients with a fixed vocal fold or with preepiglottic space involvement; however, cartilage invasion or extralaryngeal spread (T4) would be a clear contraindication (Tables 2 and 3). More studies should be done before this technique can be performed safely.

This study will continue with the hope that more patients will be enrolled and longer follow-up will be available.

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QUANTITATIVE ENDOSCOPY: INITIAL ACCURACY MEASUREMENTS

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The geometric optics of an endoscope can be used to determine the absolute size of an object in an endoscopic field without knowing the actual distance from the object. This study explores the accuracy of a technique that estimates absolute object size from endoscopic images. Quantitative endoscopy involves calibrating a rigid endoscope to produce size estimates from 2 images taken with a known traveled distance between the images. The heights of 12 samples, ranging in size from 0.78 to 11.80 mm, were estimated with this calibrated endoscope. Backup distances of 5 mm and 10 mm were used for comparison. The mean percent error for all estimated measurements when compared with the actual object sizes was 1.12%. The mean errors for 5-mm and 10-mm backup distances were 0.76% and 1.65%, respectively. The mean errors for objects <2 mm and ≥2 mm were 0.94% and 1.18%, respectively. Quantitative endoscopy estimates endoscopic image size to within 5% of the actual object size. This method remains promising for quantitatively evaluating object size from endoscopic images. It does not require knowledge of the absolute distance of the endoscope from the object, rather, only the distance traveled by the endoscope between images.

KEY WORDS — calibration, endoscopy, microlaryngoscopy, quantitative endoscopy, subglottic lesions.

INTRODUCTION

A variety of techniques have been studied in an attempt to measure subglottic or tracheal lesions. Airway lesions have been measured by use of an endoscopic ruler,[1,2] evaluation of a leak around a known endotracheal tube size,[3] insertion of bronchoscopes of various sizes, acoustic analysis,[4-7] flow volume curves, computed tomography (CT) or magnetic resonance imaging (MRI),[8] and ultrasound.[9,10] These techniques are limited by their potential for airway trauma, their cumbersome nature, patient need for irradiation, the variation of patient airway size with respiration, cost, or time to perform the study.

Endoscopy plays an important role in the diagnosis and treatment of patients with a variety of medical problems. It can give valuable qualitative information, including the appearance, extent, location, and relative size of a particular disease process. Obtaining quantitative information from endoscopic images would be advantageous to the physician, but clinical endoscopes, which contain wide-angle lenses that enable the physician to view large areas of mucosa rapidly, introduce a peripheral distortion that presents a problem in making endoscopic measurements.[11]

This peripheral distortion has been characterized by different methods. Doolin and Strande[12] were able to characterize it by calibrating an endoscope. A variance constant was used to estimate image size based on the distance of the image from the center of the field at a known distance between the endoscope and the object being measured.[12,13] An alternate method corrects for distortion mathematically by converting image points from polar to Cartesian coordinates.[14,15] Both of these methods of characterizing the distortion provide results to within 7% of the actual object size, but require that the distance between the tip of the endoscope and the object be known. A new method, quantitative endoscopy (QE), also characterizes the peripheral distortion (Vietti et al, unpublished observations). It involves obtaining 2 images of the same object, with a known distance traveled between the 2 images. There is a mathematical model underlying QE that indicates promising utility even when the absolute distance of an object from the tip of the endoscope is not available. The objective of this ongoing study is to determine the repeatability of this technique in computing absolute object size. If repeatability is demonstrated, the theoretical basis and initial observations will be validated. The validation of QE repeatability provides justification for taking further research steps to refine this technique for clinical utility.

MATERIALS AND METHODS

A 4-mm Storz-Hopkins model 27005A endoscope with an Olympus double-chip camera is calibrated (Vietti et al, unpublished observations). Figure 1 shows the system with the terminology used in this manuscript (standoff distance is the distance of the object from the tip of the endoscope; backup distance is the distance traveled by the endoscope between 2

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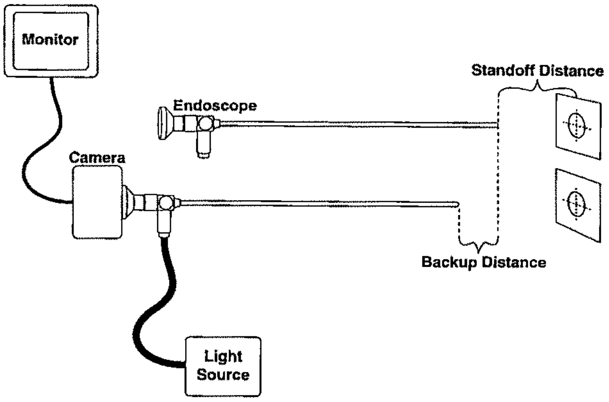

Fig 1. Endoscopic system showing standoff and backup distances.

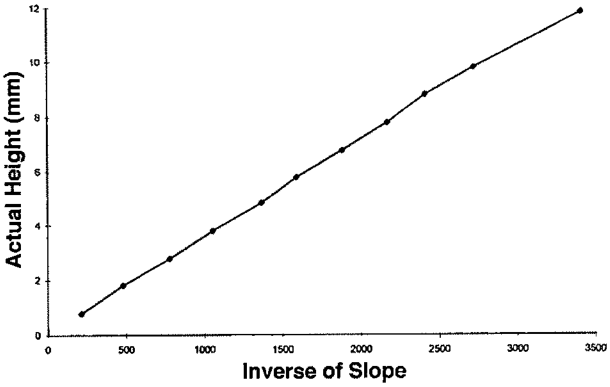

Fig 3. Endoscopic calibration line. Actual object height versus inverse of slope for each object ($r^2 = 0.9985$, slope = 0.003513', y-intercept = 0.1158).

images). The calibration involves recording images of known diameters from known standoff distances of 5.00, 10.00, 15.00, 20.00, and 25.00 mm, with verification by Vernier calipers. A VCA-CTV (Willow Peripherals, PV1026-010) video capture card captures the images from the videocassette and transfers them onto a 33-MHz IBM personal computer.

The file is imported into Microsoft Imager (Microsoft Co), where the contrast is adjusted to sharpen image edges for edge detection, and the raster images are then converted to vector format by a vectorization program (KVEC Version 1.7, KK Software, Wieden, Germany). This allows the images to be measured by a computer-assisted design (CAD) program, AutoCad LT (Rev. 13, Autodesk, Inc). The data are analyzed on a Microsoft Excel spreadsheet. The image height is plotted as a function of the standoff distance and produces a distinct hyperbola for each object height (Fig 2A) as predicted by the mathematical model for simple lenses. It is common practice in elementary physics courses to plot hyperbolic data by "mapping" them in straight-line form. This "mapping" is accomplished by substituting the inverse of the object height for the actual height to achieve a straight line. The inverse of the image height is plotted against the standoff distance to produce straight lines. Each of these lines has its own particular slope (Fig 2B). Each slope is unique to the object being measured in the field, and in mathematical formality, each slope is the inverse of the constant of proportionality of the hyperbolic relationship for each object. Next, the actual object size is plotted against the inverse of the slope for each object to produce the "endoscopic calibration line," as shown in Fig 3. The equation that represents this line ($r^2 = 0.9985$, slope = 0.003513, y-intercept = 0.1158) is unique to this particular endoscope and camera system. With this equation, object sizes can be estimated by knowing the size of 2 endoscopic images and knowing the distance traveled between these images.

This calibrated endoscope was used to estimate the sizes of 12 computer-generated circles, measuring from 0.78 to 11.80 mm, at unknown standoff distances, but at a known distance of 5.00 mm or 10.00 mm between measurements (backup distances). Figure 4 illustrates the appearance of an object through

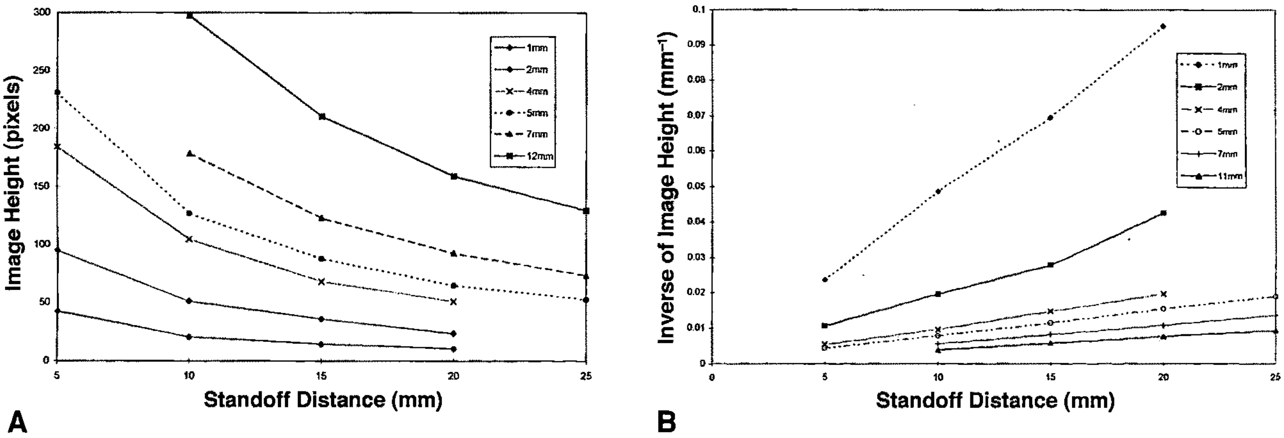

Fig 2. Calibration of endoscopic system. A) Image height versus standoff distance. Each object is represented by its own hyperbola. B) Inverse of image height versus standoff distance. Each object is represented by line with its own unique slope.

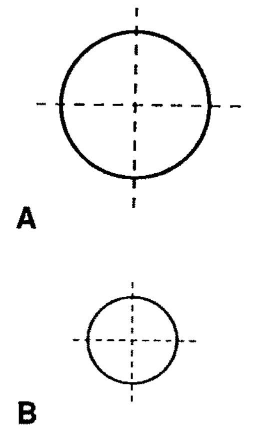

Fig 4. A) Appearance of object as seen through endoscope. B) Appearance of same object after moving endoscope known backup distance.

an endoscope, and its appearance after moving the endoscope a known backup distance. The image concentrically becomes smaller as the endoscope moves further away from the object. The QE process was evaluated as follows. One hundred sixteen additional sets of images were analyzed by the same technique used to calibrate the system. The circles were visually centered in the endoscopic field by use of a target on the monitor. Vernier calipers accurately measured the backup distances and confirmed the actual sizes of the circles under an operating microscope. The changes in the inverse of the image sizes divided by the changes in the distance from the objects gave the slope of the endoscopic calibration line. Object sizes were estimated with the equation for this line (Fig 3).

RESULTS

The percent errors of actual object sizes versus estimated object sizes for each object measured at 5-mm and 10-mm backup distances are presented in Table 1. Percent errors range from –2.42% to 4.83%.

TABLE 1. ACTUAL OBJECT SIZE VERSUS ESTIMATED OBJECT SIZE AT 5-MM AND 10-MM BACKUP DISTANCES

	5-mm Backup		*10-mm Backup*	
Actual Size (mm)	*Estimated Size (mm)*	*Error (%)*	*Estimated Size (mm)*	*Error (%)*
0.78	0.78	–0.51	0.82	4.49
1.20	1.17	–2.42	1.23	2.08
1.80	1.86	3.33	1.89	4.83
2.78	2.85	2.52	2.89	4.06
3.79	3.81	0.47	3.75	–1.06
4.82	4.89	1.35	4.86	0.83
5.78	5.81	0.45	5.82	0.74
6.77	6.73	–0.56	6.86	1.27
7.78	7.73	–0.60	7.79	0.10
8.79	8.83	0.46	8.76	–0.31
9.76	10.20	4.47	9.77	0.13
11.80	12.23	3.68	12.20	3.39

The mean percent errors and standard deviations for all object measurements are presented in Table 2. The errors are broken down by backup distance and size. The mean error for all measurements is 1.12%. Objects of <2 mm have a mean error of 0.94%, and objects of ≥2 mm have a mean error of 1.18%. The mean error for objects with a 5-mm backup distance is 0.76%, and with a 10-mm backup distance is 1.65%. For objects measuring <2 mm, the mean error when using a 5-mm backup distance is –0.53%, and the mean error when using a 10-mm backup distance is 4.03%. For objects of ≥2 mm, the mean error for a 5-mm backup distance is 1.25%, and the mean error using a 10-mm backup distance is 1.08%.

DISCUSSION

Endoscopy is a useful addition to the techniques used for the examination of patients. It gives information about the color, texture, composition, and extent of a lesion, but is limited by its inability to make absolute object size measurements. Margulies et al[16] showed a 29% average error when no measuring device was used with flexible colonoscopy, and a 13% average error if an endoscopic ruler (open-cup forceps) was used. Their measurements consistently underestimated actual object size. It was also found that there was no difference in the performance levels of experienced gastroenterologists, fellows in training, and untrained residents.[16]

The ideal method of making endoscopic measurements is an easy, safe, and reliable method with the capability of giving immediate quantitative information. Quantitative endoscopy is an appealing technique because no external reference markers need to be inserted into the endoscopic field. It utilizes the physics of a simple lens system to accurately estimate actual object size. This method does not require

TABLE 2. MEAN PERCENT ERRORS AND STANDARD DEVIATIONS FOR ALL OBJECTS MEASURING FROM 0.78 TO 11.80 MM

	N	*Mean Error (%)*	*SD (%)*
All measurements	116	1.12	8.45
<2 mm	28	0.94	14.00
5-mm backup	19	–0.53	15.80
10-mm backup	9	4.03	9.18
2 mm to 12.20 mm	88	1.18	5.79
5-mm backup	50	1.25	6.88
10-mm backup	38	1.08	4.03
5-mm backup	69	0.76	10.00
10-mm backup	47	1.65	5.40

Comparisons are made between objects <2 mm and ≥2 mm, as well as between 5-mm and 10-mm backup distances.

any additional instrumentation to be inserted into the endoscopic field, and only requires the capture of 2 images with a known distance traveled by the endoscope between these images to estimate absolute object size. The standoff distance is not needed.

Objects of <2 mm have higher standard deviations when compared to objects of ≥2 mm. This can largely be explained by the edge detection process. The edges of smaller objects are technically more difficult to detect than those of larger ones. The diameter of the endoscope used in this study was 4 mm, and the images of objects of <2 mm occupied much less than half of the endoscopic field. The difficulty in edge detection was overcome by taking multiple measurements, and the mean percent error was 0.90%. Objects of >12.20 mm were not measured, because this size is not representative of pediatric airway lesions.

A 10-mm backup distance was shown to have given more accurate estimates than a 5-mm backup distance. Each object had its own unique slope when its image height was plotted against the standoff distance. A larger backup distance should give a more accurate representation of the slope, and thus a more accurate estimation of the object size. The average errors for objects of ≥2 mm, measured at 5 mm and 10 mm, were 0.76% and 1.65%, but the standard deviations were significantly different (10.0% and 5.40%, respectively). The backup distance was not significant for objects of <2 mm. Objects of ≥2 mm were more accurately estimated with a larger backup distance. The difficulty in the edge detection of a small image after a large backup distance likely negates the linear advantage of the large backup distance. The largest backup distance that gives 2 clear endoscopic images of an entire lesion will give the best results.

We have found that the error of measurement using a roughly centered image (as the first of the 2) that nearly occupies the entire endoscopic field is comparable to measurements taken when the initial image occupies only half of the field of view. But because of the wide-angle nature of the lens, it becomes increasingly difficult to detect the edges at the extreme periphery of the endoscopic field, since the image becomes slightly unfocused. We recommend that the images be roughly centered, and that the first image occupy 70% or less of the endoscopic field.

The depth of field of the endoscope (Storz 27005A) and camera system is important, because refocusing the camera between measurements will change its calibration. This particular system has a depth of field of between 8.5 and 34.5 mm standoff distance. We recommend that the camera be focused and white-balanced 10 to 30 mm from a black-and-white object prior to starting the procedure. No further focusing should be done. Once the endoscope and camera system is calibrated, it is easy to make measurements. The system no longer needs to be calibrated, as long as the same camera and endoscope system is used.

With the implementation of certain computer software, it would be possible to get immediate quantitative results with QE. This would include automatic edge detection software and a program that could measure the image and estimate the object size. Automatic edge detection software already exists that would be able to replace the current method of transferring data to a computer-aided design program where measurements are made.

Currently, our institution is investigating further the accuracy of QE with the same and other endoscopes, including flexible nasopharyngoscopes. All clinical endoscopes are wide-angle lens systems and should be similarly characterized. Each endoscope will need its own unique endoscopic calibration line. We are planning to investigate QE for measuring subglottic lesions.

This technique of measuring 2 endoscopic images with a known backup distance can be used to estimate object size, and is most accurate for objects measuring between 2 and 12.20 mm. With improved optics and software, this technique will become increasingly more accurate. Quantitative endoscopy could be applied to any endoscopic equipment to give relatively safe, accurate, reliable, and rapid measurements.

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INTERNATIONAL CONFERENCE ON NEWBORN HEARING SCREENING DIAGNOSIS AND INTERVENTION

The International Conference on Newborn Hearing Screening Diagnosis and Intervention will be held in Milan, Italy, October 12-14, 2000. In Europe, contact Dr Ferdinando Grandori, Center of Biomedical Engineering, Polytechnic of Milan, piazza Leonardo da Vinci 32, I-20133 Milan, Italy; fax +39.02.2399.3360; e-mail NHS2000@biomed.polimi.it. In the United States, contact Dr Deborah Hayes, The Children's Hospital, 1056 E 19th Ave, B030, Denver, CO 80218; telephone (303) 861-6424; fax (303) 764-8220; e-mail: Hayes.deborah@tchden.org.

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INVOLVEMENT OF CARBON MONOXIDE IN THE INNERVATION OF THE CANINE CERVICAL ESOPHAGUS AND TRACHEA

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We investigated the involvement of carbon monoxide (CO) in the innervation of the canine cervical esophagus and trachea by means of immunohistochemistry using an antiserum against heme oxygenase–2 (HO-2). We detected HO-2–immunoreactive nerve fibers around the blood vessels and submucosal glands of the esophagus and trachea. We found HO-2–immunoreactive neurons in ganglia in the trachea and in the myenteric plexus of the esophagus. These results suggest that CO is involved as a neurotransmitter in the innervation of the canine esophagus and trachea.

KEY WORDS — carbon monoxide, dog, esophagus, heme oxygenase–2, trachea.

INTRODUCTION

Nitric oxide (NO) is now established as a transmitter in the neurotransmission in the peripheral and central nervous systems.[1,2] Carbon monoxide (CO), which shares many properties with NO, has recently been proposed as another messenger molecule.[3] However, there have been few reports regarding the involvement of CO in the innervation of the esophagus and trachea.

In the present study, we investigated the localization of heme oxygenase–2 (HO-2), the enzyme synthesizing CO, in the canine cervical esophagus and trachea by means of an immunohistochemical staining technique using antiserum against HO-2 to evaluate the possible involvement of CO in the cervical esophageal and tracheal innervation.

MATERIAL AND METHODS

Four mongrel dogs were used in the present study. All animals used in the present study were treated according to the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Experiments were also approved by the Committee of Animal Research, Kyoto Prefectural University of Medicine. Under deep anesthesia induced by intraperitoneal administration of pentobarbital sodium (30 mg/kg), the dogs were perfused with 1,000 mL of 0.1-mol/L phosphate buffer (PB) with 2% paraformaldehyde, and the cervical esophagus and trachea were removed. The extirpated esophagus and trachea were postfixed for 4 hours at 4°C in the same fixative, immersed overnight in 0.1-mol/L PB containing 15% sucrose, and sectioned on a cryostat (20 μm thick). Sections collected on gelatin-coated glass slides were provided for HO-2 immunohistochemistry.

The mounted sections were treated with 1% bovine serum for 1 hour and incubated with rabbit anti–HO-2 serum (1:1,000, StressGen Biotechnologies Corp, Victoria, Canada) for 48 hours at 4°C. The sections were then incubated with biotinylated anti-rabbit IgG (1:1,000, Vector Laboratories, Inc, Burlingame, Calif) for 1 day and with avidin-biotin-peroxidase complex (1:100, Vector Laboratories, Inc) for 1 day at 4°C. Sections were washed with 0.1-mol/L phosphate buffered saline between the steps of the reaction. Bound peroxidase was visualized in 50-mmol/L Tris hydrochloride buffer (pH 7.5) containing 0.01% 3,3' diaminobenzidine and 0.01% hydrogen peroxide. Finally, tissues were dehydrated in a graded series of ethanol-water mixtures, cleared in xylene, and covered with Entellan (Merck, Darmstadt, Germany).

RESULTS

Some HO-2–like–immunoreactive nerve fibers were observed to be clearly associated with blood vessels in the esophagus and trachea. This association had no relation to the size of the artery or vein. We also found HO-2–like immunoreactivity in arterial endothelium (see Figure, A). We found HO-2–like–immunoreactive nerve fibers in the glandular region of the esophagus and trachea, although immunoreactive nerve fibers were few in the tracheal

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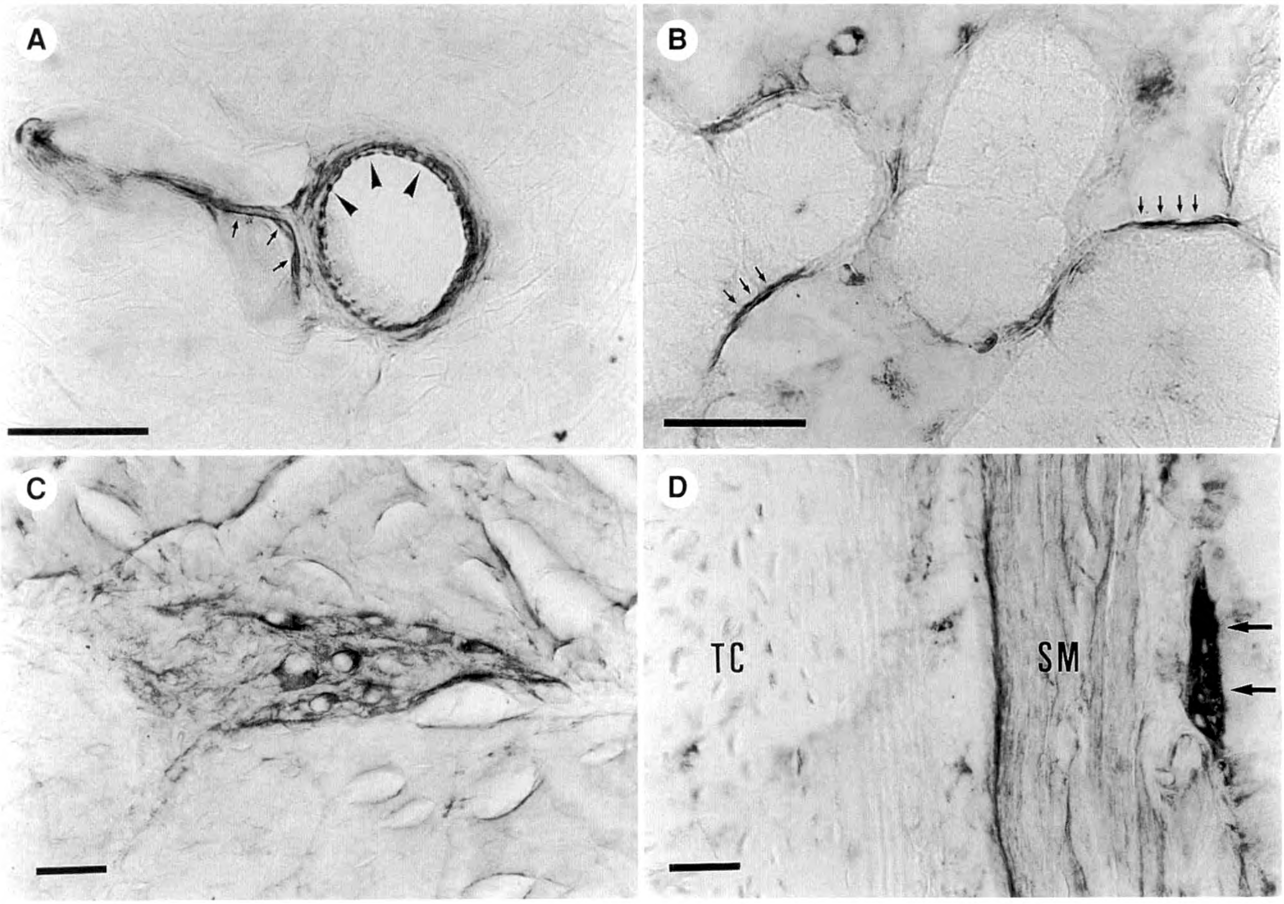

Findings. Bars — 50 μm. A) Blood vessel in submucosa of esophagus. Shows HO-2–like–immunoreactive nerve fibers (arrows). HO-2–like immunoreactivity is also detected in endothelium (arrowheads). B) Submucosal gland in esophagus. Some HO-2–like–immunoreactive nerve fibers appear to terminate in glandular cells (arrows). C) Myenteric plexus of esophagus. Some neurons are HO-2–like–immunoreactive. D) Ganglion adjacent to smooth muscle (SM) of trachea (arrows). Most of neurons have HO-2–like immunoreactivity. TC — tracheal cartilage.

glandular region. Some of these fibers appeared to terminate in glandular cells (see Figure, B). In the epithelia of the mucosa of the esophagus and trachea, no HO-2–like–immunoreactive nerve fibers were detected in any dogs examined. We found HO-2–like–immunoreactive neurons in the myenteric plexus of the esophagus (see Figure, C). We also found HO-2–like–immunoreactive neurons in the ganglia adjacent to the smooth muscle of the trachea. In these ganglia, most of the neurons were HO-2–like–immunoreactive (see Figure, D).

DISCUSSION

Heme oxygenase catalyzes the breakdown of heme to CO and biliverdin. Heme oxygenase exists in at least 2 isoforms, designated HO-1 and HO-2. The first, HO-1, is present at very low levels in the brain and has been identified as a stress protein that is inducible by a variety of stimuli.[4-7] The second, HO-2, is widely distributed throughout the body, with high concentrations in the brain, and has been demonstrated to be constitutively expressed in neuronal cells in the central nervous system as well as the peripheral nervous system.[8-14] Verma et al[9] reported that CO, like NO, may activate soluble guanylyl cyclase, and may be a physiological regulator of guanosine monophosphate levels; this finding suggests that CO may function as a neurotransmitter.

Prabhakar et al[13] reported that glomus cells are capable of synthesizing CO, and endogenous CO appears to be a physiological regulator of carotid body sensory activity. We suggested that NO may have a sensory function in the laryngeal innervation system.[15] However, CO is considered not to be involved in the sensory innervation system of the cervical esophagus or trachea of the dog, since no free endings with HO-2–like immunoreactivity were detected in the epithelium.

In the digestive system, CO originating from neurons in the myenteric plexus has been suggested to be involved in the neuronal regulation of the lower esophageal sphincter[12,14] and anal sphincter.[16] In the present study, some neurons of the myenteric plexus in the cervical esophagus were demonstrated to be HO-2–like–immunoreactive. This result suggests that CO may have another neuronal function. Since HO-2–like–immunoreactive nerve fibers were also found to be distributed around blood vessels and glands, CO is considered to be one possible neurotransmitter that regulates blood circulation and glandular secretion in the cervical esophagus.

There have been almost no reports regarding the involvement of CO in the airway innervation system. In 1996, Undem et al[17] found evidence of HO-2 in the epithelium and smooth muscle of isolated guinea pig trachea by immunohistochemistry using an antiserum against HO-2, and addressed the hypothesis that CO is involved in the relaxation of the guinea pig trachealis. In the present study, most of the neurons in the ganglia located adjacent to the tracheal smooth muscle were HO-2–like–immunoreactive, although few HO-2–like–immunoreactive nerve fibers were found in the glandular region. These results support the hypothesis of Undem et al[17] as stated above. Carbon monoxide may also be involved in the relaxation of the cervical trachea of the dog.

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COMPARATIVE HISTOLOGY OF THE MACULAE FLAVAE OF THE VOCAL FOLDS

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A light and electron microscopic comparative investigation of the maculae flavae of the vocal folds was carried out on excised human and canine adult larynges. The structure and functions of human adult maculae flavae (HMF) were found to differ from those of canine adult maculae flavae (CMF). The maculae flavae were composed of fibroblasts, elastic and collagenous fibers, and ground substance in humans and canines. The density of fibroblasts in HMF was found to exceed that in CMF. Fibroblasts in HMF were stellate with processes or spindle-shaped, and the nucleus-cytoplasm (N/C) ratio was small. Rough endoplasmic reticulum and Golgi apparatus were well developed in the cytoplasm. Fibroblasts in CMF were oval, and the N/C ratio was large. Endoplasmic organs were poorly developed in the cytoplasm. Synthesized elastic and collagenous fibers were more numerous in HMF than CMF, and the density of both in HMF was much greater than that in CMF. Ground substance was more abundant in CMF than HMF. Apparently, CMF did not produce elastic and collagenous fibers in amounts sufficient to develop vocal ligaments. The HMF contributes to the formation of the vocal ligaments and the layered structure of human vocal folds.

KEY WORDS — comparative histology of larynx, histology of larynx, larynx, macula flava, vocal fold.

INTRODUCTION

Adult human vocal folds have a layered structure consisting of epithelium; superficial, intermediate, and deep layers of the lamina propria; and vocalis muscle.[1] A transition zone, consisting of intermediate and deep layers of lamina propria, is the vocal ligament.[1] Maculae flavae are located at the anterior and posterior ends of the membranous portion of the vocal fold.[1,2] The maculae flavae were previously shown to control the synthesis of fibrous components such as the vocal ligaments.[3-5]

Maculae flavae are also present at the anterior and posterior ends of the membranous portion of animal vocal folds.[6,7] But there is no structure equivalent to the human vocal ligament.[6,7] If the maculae flavae control the synthesis of fibrous components such as the vocal ligament of the human vocal fold, the structure and/or functions of the human maculae flavae should differ from those of animals.

We compared the structure of the maculae flavae of the vocal folds in human beings and dogs by light microscopy (LM) and transmission electron microscopy (TEM), and herein discuss their functions.

MATERIALS AND METHODS

Five normal human adult larynges obtained from autopsy cases and 5 normal canine adult larynges were used.

For LM, the specimens were fixed in 10% formalin, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Hematoxylin and eosin and elastica–van Gieson staining was carried out on each section.

For TEM, the specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution, and postfixed in 2% osmium tetroxide with cacodylate buffer solution at 4°C for 2 hours. After rinsing with cacodylate buffer solution, the specimens were dehydrated in graded concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined by LM. Thin sections were produced with an ultramicrotome and stained with uranyl acetate and lead citrate, and tannic acid for elastin, followed by a JEM-2000EX TEM observation.

RESULTS

The maculae flavae were located at the anterior and posterior ends of the human adult and canine adult vocal folds. The anterior macula flava was connected to the thyroid cartilage via an intervening anterior commissure tendon. The posterior macula flava was attached to the vocal process of the arytenoid cartilage posteriorly.

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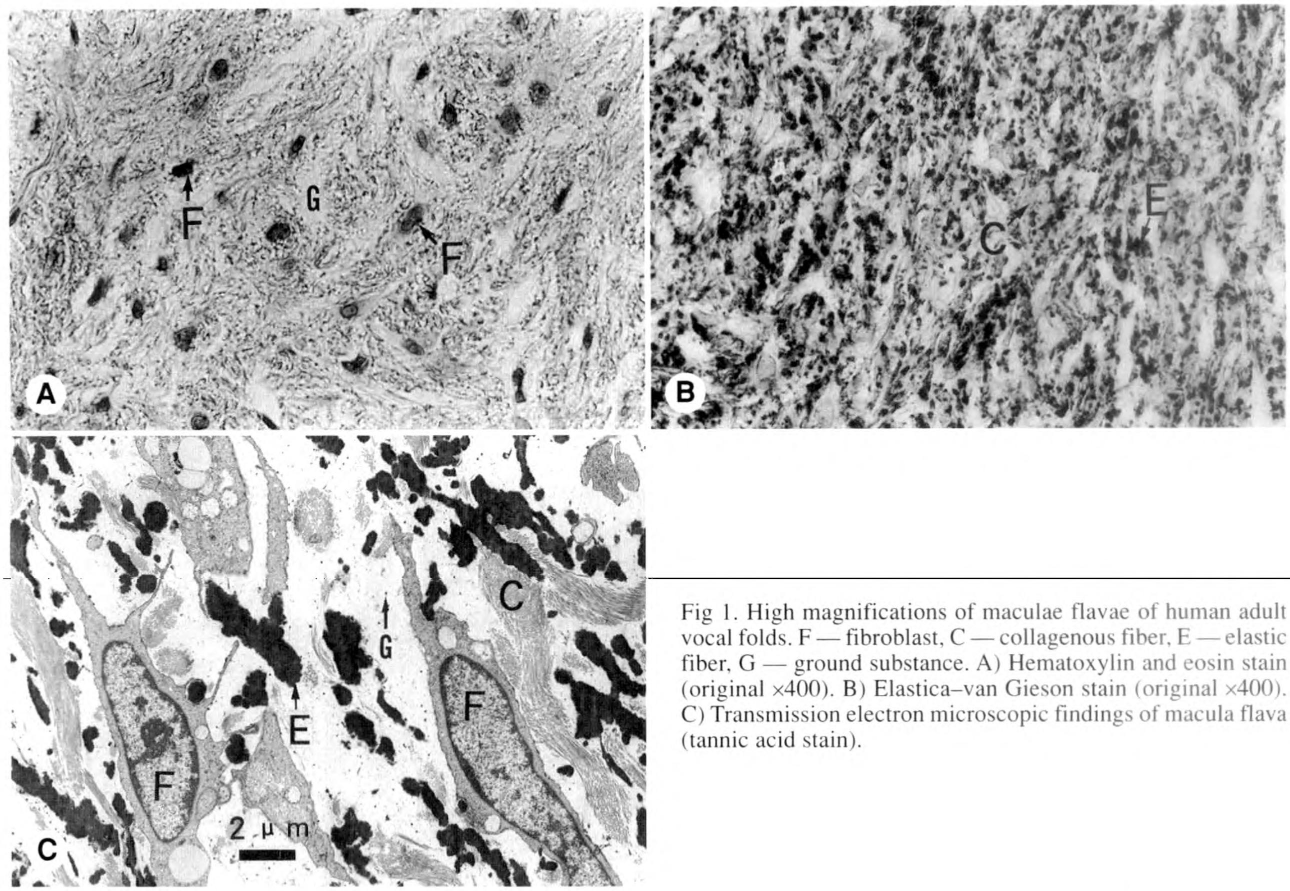

Fig 1. High magnifications of maculae flavae of human adult vocal folds. F — fibroblast, C — collagenous fiber, E — elastic fiber, G — ground substance. A) Hematoxylin and eosin stain (original ×400). B) Elastica–van Gieson stain (original ×400). C) Transmission electron microscopic findings of macula flava (tannic acid stain).

In human adult vocal folds, the maculae flavae were composed of dense masses of fibrous tissue. They were elliptical in shape and approximately 1.5 × 1.5 × 1 mm in size. The vocal ligament was situated between the anterior and posterior maculae flavae.

In canine adult vocal folds, the maculae flavae were vague masses of fibrous tissue. They were tortuous in shape and approximately 1.5 × 1.5 × 1 mm in size. No vocal ligaments could be detected between the anterior and posterior maculae flavae.

Figures 1 and 2 present at high magnification maculae flavae of human adult and canine adult vocal folds, composed in either case of fibroblasts, elastic fibers, collagenous fibers, and ground substance.

Fibroblasts. Numerous fibroblasts could be seen in the maculae flavae of both species (Figs 1A,C and 2A,C). The density of fibroblasts in the human adult maculae flavae was higher than that of the canine adult maculae flavae (Figs 1A,C and 2A,C).

In the human adult maculae flavae, the fibroblasts were stellate with processes or spindle-shaped, about 5 × 10 μm in size, and the nucleus-cytoplasm ratio was small (Fig 3A). Rough endoplasmic reticulum and Golgi apparatus were well developed in the cytoplasm of the fibroblasts (Fig 3A). The fibroblasts were in an active, not resting, state. Other organs in the cytoplasm were mitochondria, smooth endoplasmic reticulum, and vesicles. Lipid droplets were sometimes seen in the cytoplasm (Fig 3A).

In the canine adult maculae flavae, the fibroblasts were oval and 3 × 5 μm in size (Fig 3B), ie, smaller than the human adult maculae flavae, and the nucleus-cytoplasm ratio was larger than in the latter. Endoplasmic organs such as rough endoplasmic reticulum and Golgi apparatus were poorly developed in the cytoplasm of the canine fibroblasts (Fig 3B), which were less active than in the human adult vocal folds. Lipid droplets could not be seen in the cytoplasm.

Elastic Fibers. Elastic fibers were noted to consist of microfibrils and amorphous substances in the human adult and canine adult maculae flavae. The density was greater in the former than in the latter (Figs 1B,C and 2B,C).

Figure 4A shows the mode of synthesis of elastic fibers in canine adult maculae flavae. Some vesicles were present along the periphery of cytoplasm of the fibroblasts. Newly released amorphous materials were present on the cell surface. Microfibrils could be seen situated around the amorphous material. Microfibrils had assembled on which elastin ap-

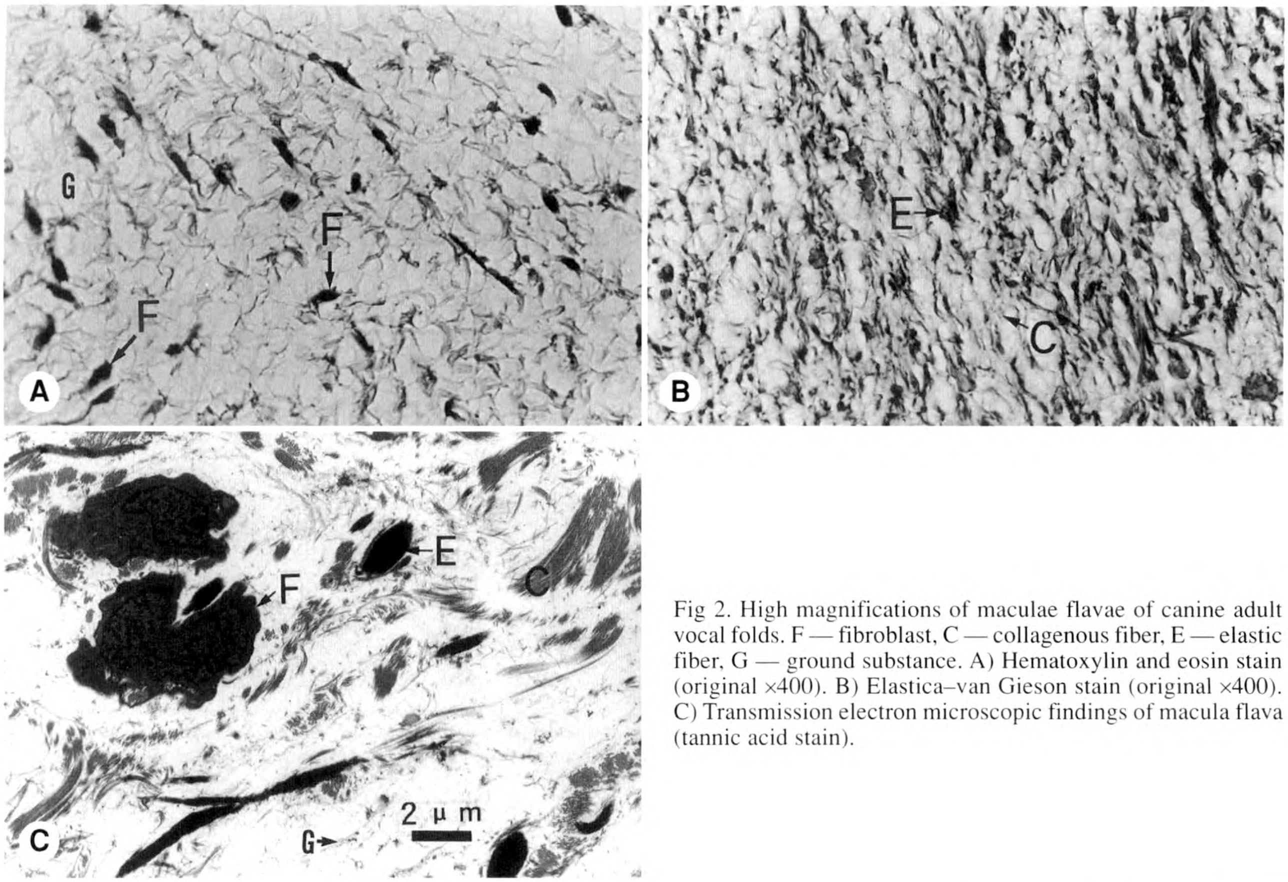

Fig 2. High magnifications of maculae flavae of canine adult vocal folds. F — fibroblast, C — collagenous fiber, E — elastic fiber, G — ground substance. A) Hematoxylin and eosin stain (original ×400). B) Elastica–van Gieson stain (original ×400). C) Transmission electron microscopic findings of macula flava (tannic acid stain).

peared to be deposited. Amorphous substances of the elastic fibers apparently had been produced by the fusion of microfibrils.

The synthesis of elastic fibers in the canine adult maculae flavae occurred in the same manner as in the human adult maculae flavae. The synthesized elastic fibers were more numerous in the human adult maculae flavae (Figs 1B,C and 2B,C).

Collagenous Fibers. Collagenous fibers were composed of numerous collagenous fibrils and were 40 to 50 nm in width in both species. The density of the collagenous fibers in the human adult maculae flavae was greater than that in the canine adult maculae flavae (Figs 1B,C and 2B,C). Figure 4B shows the synthesis of collagenous fibers in canine maculae flavae. Some vesicles were present along the periphery of the cytoplasm of canine fibroblasts. Newly released amorphous materials were present on the cell surfaces of the fibroblasts. Microfibrils 10 to 15

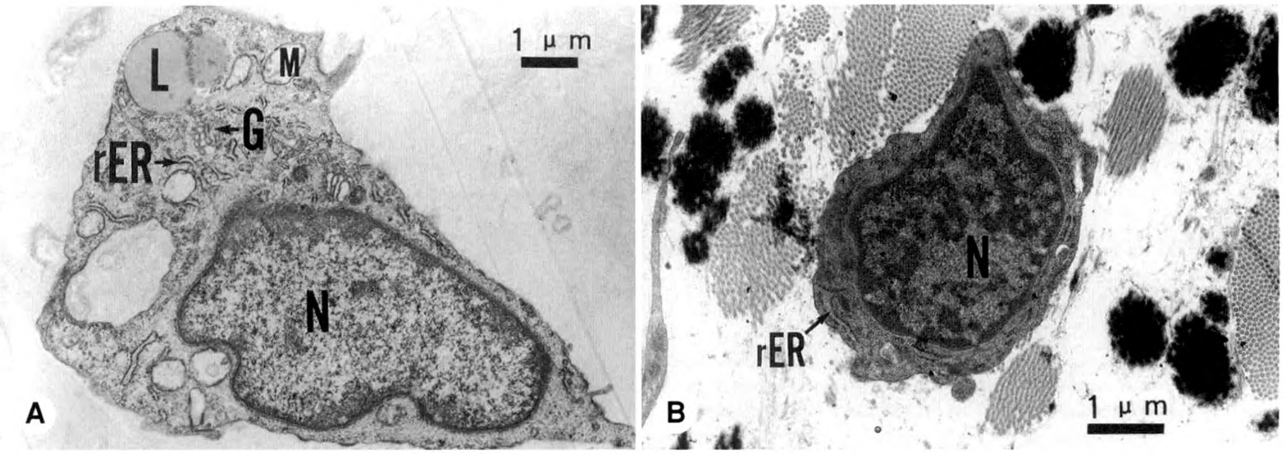

Fig 3. Transmission electron microscopic findings of fibroblasts in maculae flavae. rER — rough endoplasmic reticulum, N — nucleus. A) Human adult vocal fold (uranyl acetate and lead citrate stain). G — Golgi apparatus, M — mitochondrion, L — lipid droplet. B) Canine adult vocal fold (tannic acid stain).

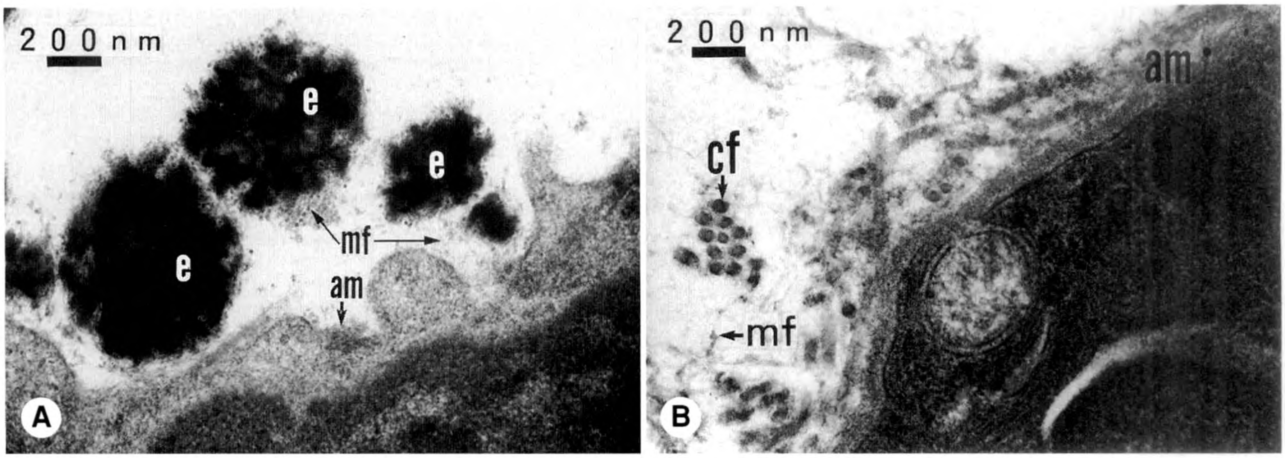

Fig 4. Synthesis of fibers in canine adult macula flava (transmission electron microscopy, tannic acid stain). am — amorphous material, mf — microfibril, e — elastin, cf — collagenous fibril. A) Elastic fibers. B) Collagenous fibers.

nm in width were situated around the amorphous material. The collagenous fibrils were near microfibrils.

The synthesis of collagenous fibers in the canine maculae flavae was the same as that in the human adult maculae flavae. The synthesized collagenous fibers were more numerous in the human adult maculae flavae (Figs 1B,C and 2B,C).

Ground Substance. Ground substance was more abundant in the canine adult maculae flavae than in the human adult maculae flavae (Figs 1A and 2A). The ground substance was homogeneous or slightly flocculent in both species on electron microscopy (Figs 1C and 2C).

DISCUSSION

Lanz and Wachsmuth[8] described the anterior macula flava of the vocal fold as the nodulus elasticus in their textbook of anatomy. Hirano[1] noted masses of dense elastic fibers at the anterior (anterior macula flava) and posterior (posterior macula flava) ends of the vocal folds. Previous light and electron microscopic investigation indicated the macula flava to be a mass of dense fibrous tissue composed of fibroblasts, elastic fibers, collagenous fibers, and ground substance.[3-5]

Maculae Flavae of Human Vocal Folds. From the physiological and biomechanical points of view, the maculae flavae of the human vocal folds have very important functions. Extracellular matrices, including fibers, are produced by fibroblasts. The fibroblasts in the maculae flavae are denser than in Reinke's space of the vocal folds,[9] and much more actively produce elastic and collagenous fibers than those in Reinke's space of adults and newborns.[9] The maculae flavae synthesize elastic and collagenous fibers and control the synthesis of fibrous components such as the vocal ligaments.[3] The maculae flavae are particularly essential to the growth, development, and aging of the vocal folds.[3-5]

Newborn vocal folds have no structure that could be considered a vocal ligament.[1,2,4] The fibrous components are not as abundant,[4] and the fibroblast density is greater than that of human adult maculae flavae.[4] The numerous fibroblasts actively start to form elastic and collagenous fibers.[4]

The vocal ligaments are mature after puberty.[10] The maculae flavae of the vocal folds contribute to the development and metabolism of the vocal ligaments.[3-5,9]

In aged human adults, the number of fibroblasts decreases and components in the cytoplasm, such as the Golgi apparatus and rough endoplasmic reticulum, are fewer than at younger ages.[5] Some fibroblasts and components in the cytoplasm degenerate.[5] The numbers of collagenous and elastic fibers synthesized by fibroblasts decrease.[5] The decrease in the number and activation of fibroblasts in the maculae flavae indicates decreased synthesis of fibrous components in the vocal fold mucosa,[5] with a consequent effect on extracellular matrices of vibrating tissue and some contribution to voice aging.[5]

Maculae Flavae of Canine Vocal Folds and Comparison With Human Maculae Flavae. Maculae flavae are present at the anterior and posterior ends of the membranous portion of animal vocal folds[6,7] and are composed of fibroblasts, elastic and collagenous fibers, and ground substance. There is no structure equivalent to the human vocal ligament or the layered structure of the human folds.[1,6,7] The structure and roles of the maculae flavae of human vocal folds may possibly differ from those of animal vocal folds.

Human newborn vocal folds have no structure that could be considered to be a vocal ligament,[1,2,4] and the fibrous components are fewer in the maculae flavae.[4] The structure of the maculae flavae is similar to that of canine adult maculae flavae. But the newborn fibroblast density in the maculae flavae is great, and numerous fibroblasts actively start to form elastic and collagenous fibers.[4]

In the human adult vocal fold, the structure and functions of the maculae flavae were found in this study to differ from those of canine adult maculae flavae. The maculae flavae were composed of fibroblasts, elastic fibers, collagenous fibers, and ground substance in both species. The human adult maculae flavae were composed of a dense mass and were elliptical in shape, while the canine adult maculae flavae consisted of a vague mass and were tortuous in shape. The most characteristic difference was the shape of the fibroblasts. The fibroblasts in the human maculae flavae were stellate with processes or spindle-shaped, and the nucleus-cytoplasm ratio was small. The rough endoplasmic reticulum and Golgi apparatuses were well developed in the cytoplasm, indicating the fibroblasts to be in the active phase and producing many intercellular matrices such as collagenous and elastic fibers and ground substance. The fibroblasts in canine adult maculae flavae were oval, and the nucleus-cytoplasm ratio was large. The endoplasmic organs were poorly developed in the cytoplasm, indicating the fibroblasts not to be in the active phase nor producing many intercellular matrices. The density of fibroblasts in the human maculae flavae was greater than that in the canine adult maculae flavae. Synthesized elastic and collagenous fibers were thus more numerous in the human maculae flavae. The elastic and collagenous fiber density in the human maculae flavae was much higher than that in the canine adult maculae flavae. The canine maculae flavae did not appear to produce elastic and collagenous fibers in amounts sufficient to develop vocal ligaments or a layered structure of the vocal folds.

Human adult maculae flavae differ from canine adult maculae flavae morphologically, according to the present results. Human maculae flavae contribute to the development and metabolism of the vocal ligament and extracellular matrices in the human vocal folds.

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MICROSURGERY OF SULCUS VERGETURE WITH CARBON DIOXIDE LASER AND INJECTABLE COLLAGEN

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Between January 1989 and June 1998, we operated on 45 patients for sulcus vergeture. The studied population encompassed 38 women (84%) and 7 men (16%). The median age was 36 (range 12 to 71 years). The surgical technique is based on a concept of Cornut and Bouchayer according to which the dissection of the epithelium adherent to the deep subepithelial plane improves the vocal fold vibration. Dissection is performed with a single-pulsed carbon dioxide laser at 2 to 3 W with a pulse duration of 0.1 second. We use the Super-pulse microwave. The Acuspot micromanipulator provides a spot size of 250 μm at 350-mm focal length. When the vocal fold is atrophic, surgery is completed with a bovine or autologous collagen injection; the median injected quantity is 0.3 mL (range 0.1 to 0.4 mL). The epithelial microflap is redraped with fibrin glue. Voice therapy is indispensable for correcting the associated hyperkinetic dysphonia. The median postoperative follow-up period is 5 months (range 1 to 18 months). In terms of median values, the maximum phonation time improved from 9 to 13 seconds, the phonation quotient improved from 296.5 to 228.5 mL/s, and the spectral analysis distribution improved by 1 class. Stroboscopic examination reveals an improvement of the vibratory symmetry, amplitude, and wave. Subjectively, the patients describe an improved ability for vocal effort and the regression or disappearance of vocal fatigue. Although the timbre is improved, the voice often remains breathy and hoarse.

KEY WORDS — carbon dioxide laser–assisted phonomicrosurgery, injectable collagen, sulcus vergeture, vocal fold atrophy.

INTRODUCTION

Sulcus vergeture resembles the shape of an orange segment; the bottom lining of its mucosa is extremely

[redacted]

was an acquired condition (Garel, Van Caneghem).[2] The same discussion still remains between authors supporting a congenital causation[1,7] and supporters with type III thyroplasty can also be performed.[17] Pontes and Belhau[18] use an original technique that consists of multiple transverse incisions along the vocal fold; the technique is reported as successful.

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tified, it is removed during the same surgical procedure (prior to the microsurgical approach of the sulcus vergeture). Following this careful examination, the free edge of the vocal fold, which displays the

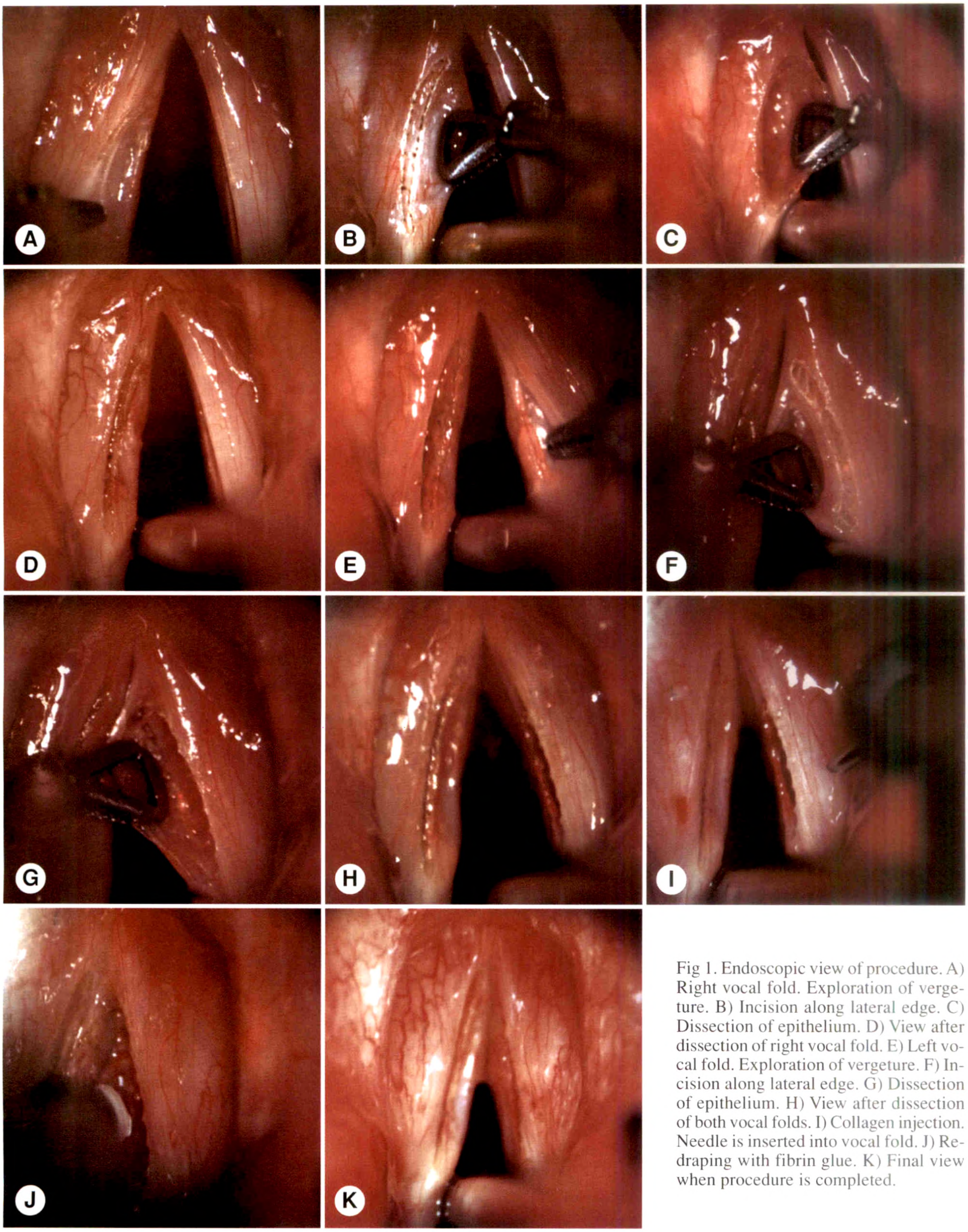

Fig 1. Endoscopic view of procedure. A) Right vocal fold. Exploration of vergeture. B) Incision along lateral edge. C) Dissection of epithelium. D) View after dissection of right vocal fold. E) Left vocal fold. Exploration of vergeture. F) Incision along lateral edge. G) Dissection of epithelium. H) View after dissection of both vocal folds. I) Collagen injection. Needle is inserted into vocal fold. J) Redraping with fibrin glue. K) Final view when procedure is completed.

to resume work 1 to 2 months after surgery.

Preoperative and postoperative parameters were compared by a Wilcoxon signed rank test. A Spearman coefficient was used to assess the correlation between postoperative parameters and the length of follow-up.

RESULTS

The immediate postoperative period of each pa-

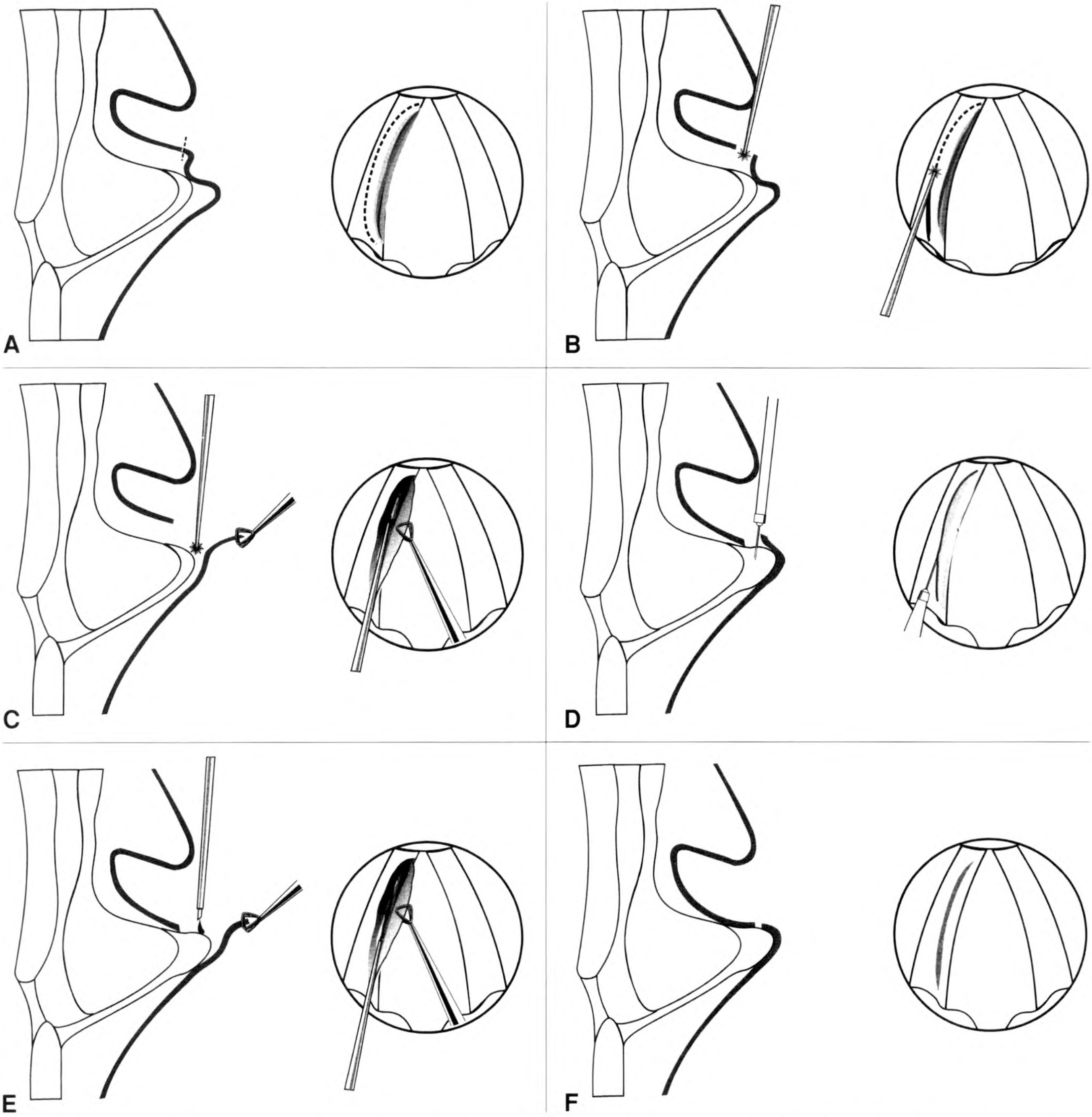

Fig 2. Drawings of procedure. A) Incision line along lateral and superior edge of vergeture. B) Carbon dioxide laser incision, 250-µm beam, Super-pulse, single pulse, 0.1 second, 3 W. C) Carbon dioxide laser dissection between epithelium and vocal ligament, with same parameters, and traction of epithelium to midline. D) Collagen injection into vocal ligament. E) Redraping of epithelium with fibrin glue. F) Final view.

tient was straightforward: no untoward incident directly related to surgery was observed. Following the CO_2 laser–assisted dissection and the collagen injection, we did not observe any epithelial necrosis of the sulcus vergeture.

Sulcus vergeture (Table 1) affected both vocal folds in 33 cases (73%), and 1 vocal fold in 12 cases (27%). Twenty-nine cases (64%) displayed associated lesions: in 13 cases (29%), the lesions were sulcus-related (epidermoid cyst, mucosal bridge, microweb); in 10 cases (22%), the lesions were of different benign disorders (nodule, polyp, Reinke's edema, scar, granuloma). Six patients (13%) presented both types of lesions. In 31 cases (69%), sulcus vergeture was the primary diagnosis. In 14 cases (31%), sulcus vergeture was secondarily discovered or confirmed during surgery.

Apart from CO_2 laser dissection, collagen injection

TABLE 1. DISTRIBUTION OF LESIONS IN TERMS OF INCIDENCE (N = 45)

Main Lesions	*Associated Lesions*		*N*
Bilateral sulcus			13
Bilateral sulcus	Unilateral nodule		3
Sulcus			3
Bilateral sulcus	Epidermoid cyst		2
Bilateral sulcus	Mucosal bridge		2
Nodules	Bilateral sulcus		2
Bilateral Reinke's edema	Bilateral sulcus		1
Bilateral Reinke's edema	Bilateral sulcus	Mucosal bridge	1
Bilateral scar	Bilateral sulcus		1
Bilateral sulcus	Bilateral mucosal bridge		1
Bilateral sulcus	Bilateral epidermoid cyst		1
Bilateral sulcus	Epidermoid cyst		1
Bilateral sulcus	Mucosal bridge		1
Epidermoid cyst	Sulcus	Microweb	1
Granuloma	Bilateral scar	Sulcus	1
Nodules	Bilateral sulcus	Mucosal bridge	1
Nodules	Sulcus		1
Polyp	Scar	Sulcus	1
Polyp	Bilateral sulcus	Mucosal bridge	1
Polyp	Bilateral sulcus		1
Scar	Bilateral sulcus	Mucosal bridge	1
Scar	Sulcus		1
Sulcus	Epidermoid cyst	Mucosal bridge	1
Sulcus	Bilateral epidermoid cyst		1
Sulcus	Polyp		1
Sulcus	Epidermoid cyst		1

TABLE 3. STATISTICAL DIFFERENCES IN OBJECTIVE MEASUREMENTS MADE BEFORE AND AFTER TREATMENT

			Percentiles		
	N	*Mean ± SD*	*25th*	*50th (Median)*	*75th*
Maximum phonation time (s)	21	2.5 ± 4.4	0	3	5
Phonation quotient (mL/s)	20	−86.4 ± 142	−155	−42.3	2.25
Spectral analysis classification	30		0	1	1

was necessary in all cases. The median injected quantity per surgical procedure was 0.3 mL (range 0.1 to 0.4 mL). Forty-two patients were injected with bovine collagen, and 4 patients received autologous collagen. Three female patients underwent 2 injections of bovine collagen. One patient first underwent an injection of bovine collagen, followed by an injection of autologous collagen.

The median postoperative follow-up was 5 months (range 1 to 18 months).

In terms of median values, we noticed a significant improvement of the aerodynamic measurements (Table 2) as follows: from 9 to 13 seconds for the maximum phonation time; and from 296.5 to 228.5 mL/s for the phonation quotient. Those results square with a median improvement of 3 seconds for the maximum phonation time and 42.35 mL/s for the phonation quotient (Table 3).

The acoustic analysis does not show any significant modification in the fundamental frequency or in the intensity. However, in terms of median value, the spectral analysis reveals an improvement by 1 class.

No significant correlation was found between the follow-up period and the achieved result (maximum phonation time, r = .172; phonation quotient, r = .277; spectral analysis classification, r = .176; none significant). Stroboscopic examination shows that the vibration returns, approximately, 1 month after surgery. The vibratory movement first displays the return of

TABLE 2. OBJECTIVE MEASUREMENTS MADE BEFORE AND AFTER TREATMENT

	N		*Mean ± SD*		*Percentiles*						*Wilcoxon Signed Rank*
					25th		*50th (Median)*		*75th*		
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Test (p)*
Maximum phonation time (s)	21	21	11.3 ± 6.7	13.9 ± 6.7	6.5	9.5	9	13	13	17.5	.007
Phonation quotient (mL/s)	20	20	294.7 ± 176.7	208.4 ± 87.7	129	136	296.5	228.5	405	253	.012
Fundamental frequency (Hz)	30	30	211.7 ± 55.6	225.0 ± 45.1	177.5	207.5	205	230	248.7	252.5	NS
Spectral analysis classification	30	30			2	3	3	3	3	4	<.001
Modal intensity (dB)	15	15	63.0 ± 7.4	61.1 ± 6.2	57	54	63	63	70	65	NS
Minimum intensity (dB)	9	9	59.1 ± 3.6	56.1 ± 5.7	55	51.5	60	56	62	59	NS
Maximum intensity (dB)	19	19	84.1 ± 11.6	88.7 ± 9.0	74	82	81	91	95	95	NS
Intensity range (dB)	9	9	27.1 ± 6.9	33.2 ± 14.1	22	27.5	26	32	33	44.5	NS

NS — not significant.

the amplitude and then the return of the mucosal wave. The definitive appearance of the vocal fold vibration is achieved in the fifth month: the amplitude, the mucosal wave, and the symmetry are all improved. Although glottal closure is also improved, it is not normalized and remains irregular. Voice therapy, during the first 4 months, corrects the hyperactivity of the laryngeal muscles during phonation. Subjectively, the patients describe an improved phonatory ability during vocal effort and the regression or disappearance of vocal fatigue. Although the timbre is improved, the voice remains breathy and hoarse.

DISCUSSION

We propose a technique inspired directly by the

[redacted]

bovine collagen for several indications,[14] without having observed any long-term local or general side effects. However, the US Food and Drug Administration has not approved bovine collagen, and further, certain patients express fear regarding the possible transmission of bovine spongiform encephalopathy. For those reasons, autologous collagen[15] can be proposed as an alternative. The advantage of bovine collagen is its immediate availability if a further quantity is required during surgery. The advantage of autologous collagen is that it is free of immune risk. Further, whereas the telopeptides of the bovine collagen molecules have been cleaved in order to avoid an immune reaction, the autologous collagen molecules are intact. This entirety promotes period of 3 to 4 months. The aim of the therapy is to correct the inadequate compensatory hyperactivity of the laryngeal muscles that the patient has developed, often over a period of several years. Voice therapy plays the same role as physiotherapy after orthopedic surgery. The concept of Bouchayer and Cornut[3] is based on yielding an improved vocal fold vibration by releasing the fibrosis between the epithelium and the vocal ligament. To achieve this, dissection must encompass practically the entire ligamentous vocal fold. Furthermore, postoperative scarring induces fibrosis. The patient must therefore be warned clearly, before surgery, of the importance of complete compliance to the period of postoperative voice therapy. The patient must also be informed that the definitive vocal result will be achieved only after an average period of 5 months. Our median follow-up is 5 months. This does not signify that after that period, we stopped the follow-up as a matter of principle, but rather, that we did not observe further progress — objective or subjective — past that period. We then ended the regular follow-up controls and advised the patients to contact us again should a problem arise.

The interval of time needed before attaining the final postoperative result ranges between 1 and 18 months. This explains why the median follow-up period is statistically more interesting than the mean follow-up period. However, once the result was achieved, no further voice improvement was observed during the occasional remote checkups after the postoperative follow-up. Relative to the long-term outcome of the collagen injected within the vocal cord, and on the basis of Ford's[27] work and our study,[28] it is possible that the realignment of the collagen fibers and the deposition of new collagen could result in a delayed voice improvement. Thus, a statistical analysis of the voice parameters of those patients, 12 to 18 months after surgery, could prove interesting.

The patient must choose a period in his or her professional, social, and family life that will allow him or her to complete the therapeutic management. We also recommend explaining to the patient the advantages of the operation: an improved resistance during vocal effort; a reduction or even the elimination of vocal fatigue and dysesthesia; and an improved intensity control. In other words, the aerodynamic parameters show an improved vocal management. On the other hand, although the vocal quality will most probably improve (improved timbre and a less breathy and hoarse voice), the range of improvement will vary from patient to patient, and thus it is more difficult to commit oneself on that point. Usually, patients are entirely satisfied with the achieved improvement. Most patients do not wish for a "normal" voice, but for a voice that allows them to assume their professional or social obligations. They recall that since early childhood, their voice has always been hoarse without its disturbing them. This first group of patients probably belongs to the category of patients with a genuine congenital disorder. Certain patients specify that they have only recently noticed the hoarseness (6 months to 2 years), usually following professional activities. When questioned on the vocal quality before the change, they recall it as being normal. This second group of patients probably belongs to the category of patients with an acquired condition, even if that is difficult to prove. We therefore believe that both causes exist: congenital and acquired.[11]

Regarding the sex ratio, we observed a slight predominance of young women.

We wish to stress that sulcus vergeture is frequently associated with other lesions (69% in our series) and that during surgery for those lesions, sulcus vergeture can be discovered or confirmed in approximately 30% of the cases (31% in our series). We believe it is better to postpone the surgical treatment of a sulcus vergeture if the patient has not been preadvised that during surgery a sulcus vergeture could be discovered. Indeed, the patient must be made aware that the postoperative management of sulcus vergeture is much more restricting than that following surgery for simple nodules or polyps. Secondary confirmation or discovery of a sulcus vergeture also justifies suspension laryngoscopy under general anesthesia as an operative setting for phonomicrosurgery; it allows a careful exploration of the vocal folds before the actual microsurgical procedure begins.

CONCLUSION

Provided the working parameters are strictly respected, the CO_2 laser is an effective tool for treating sulcus vergeture. Injectable collagen, be it bovine or autologous, can be used to repair the atrophic vocal fold. Voice therapy is an indispensable component of the treatment and usually lasts 4 months. The limitations of this microsurgical operation must be explained clearly prior to surgery, so that the patient may have realistic expectations of the result. In regard to the latter, the patient can undoubtedly expect an improved vocal performance.

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VIDEOENDOSCOPIC LARYNGEAL SURGERY

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This paper introduces videoendoscope-assisted laryngeal surgery with office-based equipment. With this technique, a patient is seated and the nose, pharynx, and larynx are topically anesthetized. A flexible videoendoscope with a light-sensitive charge-coupled device chip built into the tip is transnasally inserted by an assistant. Specially designed fine-tipped forceps and scalpels were developed for removal of laryngeal lesions. Videoendoscopic laryngeal surgery was undertaken in 114 cases of laryngeal lesions such as polyps, granuloma, and cancer. For benign vocal fold lesions, postoperative vocal function was shown to be improved on aerodynamic and perceptual analyses. For laryngeal tumors, biopsy of the lesion was easily undertaken. Videoendoscopic laryngeal surgery presents the following advantages. It is applicable to outpatients not requiring general anesthesia, it enables functional monitoring of the patient's voice and vocal fold during phonation, it allows for delicate manipulations with both hands, and it gives high-resolution images in comparison to conventional fiberscopy.

KEY WORDS — functional monitoring, laryngeal surgery, office-based equipment, topical anesthesia, videoendoscope.

INTRODUCTION

Laryngomicrosurgery with a suspension laryngoscope under general anesthesia using an endotracheal intubation tube has been popular since its introduction in 1960.[1,2] It provides for stability of the surgical field with no gag reflex and enables precise identification of lesions. With this technique, however, an intubation tube obstructs the surgical field, and functional control cannot be achieved. Ideally, in phonosurgery, the operator would be able to evaluate the patient's voice and observe the mucosal vibration of the vocal fold during phonation. In order to overcome the disadvantages of suspension laryngomicrosurgery with an intubation tube, many different methods of endolaryngeal surgery have been tried. Indirect laryngomicrosurgery with a mirror under topical anesthesia,[3,4] indirect microlaryngostroboscopic surgery with a mirror or a rigid telescope under topical anesthesia,[5] and laryngomicrosurgery with a suspension laryngoscope under neuroleptoanalgesia (NLA)[6] have been undertaken with a regular or a strobe light source. However, these methods have not become widely used because of difficult manipulations and poor images of the surgical field.

Since 1991, Kojima et al have been carrying out fiberoptic laryngomicrosurgery with a stroboscope under local anesthesia as a day surgery using office-based equipment.[7,8] With this technique, the operator utilizes both hands freely with functional monitoring of the patient's voice and larynx, while an assistant inserts a fiberoptic endoscope transnasally and the patient pulls the tongue. The latest videoendoscope system employs a charge-coupled device (CCD) chip built into the tip to offer the convenience of a large-sized image and deliver a higher level of resolution than can a conventional fiberoptic endoscope. It is now possible to apply a videoendoscope system to the field of laryngology.[9] The present study introduces videoendoscope-assisted laryngeal surgery with office-based equipment under topical anesthesia. This technique provides for functional phonosurgery by monitoring the patient's voice and provides clear visualization of the vocal fold lesions during phonation.

EQUIPMENT

Videoendoscope System. The videoendoscope system used in this study was the EVIS-200 system (Olympus Optical Co, Ltd, Tokyo, Japan). This system consists of a flexible endoscope component, a video system center, a xenon light source, a color video monitor, a videocassette recorder, and a color video printer. For the endoscope component, a flexible videoendoscope ENF type 200, without an aspiration channel, and a flexible videoendoscope ENF type T200, which has an aspiration channel, were used. A light-sensitive CCD chip is built into the tip as an ultraminiature video camera. The tip and the

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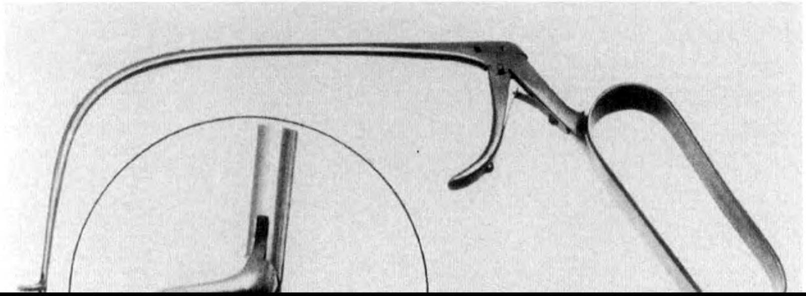

Fig 1. Specially designed fine-tipped forceps allow manipulation in plane parallel to supe-

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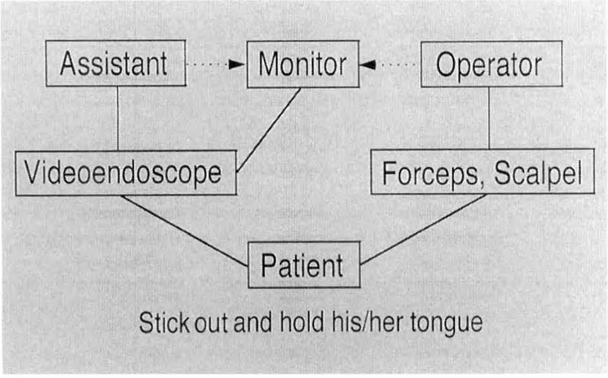

Fig 3. System for videoendoscopic laryngeal surgery.

iness, were scored as 0, 1, 2, or 3. A score of 3 was given for a severely rough voice, a score of 2 for a moderately rough voice, a score of 1 for a slightly rough voice, and a score of 0 for no rough voice. The scores given by the 3 listeners were averaged and classified again into 4 categories: scores of 0, 1, 2, or 3. The degree of breathiness was also scored as 0, 1, 2, or 3 in the same way.

RESULTS

Videoendoscope-assisted laryngeal surgery under topical anesthesia was accomplished in 104 of the 114 cases (91%). In 10 cases, the operation could

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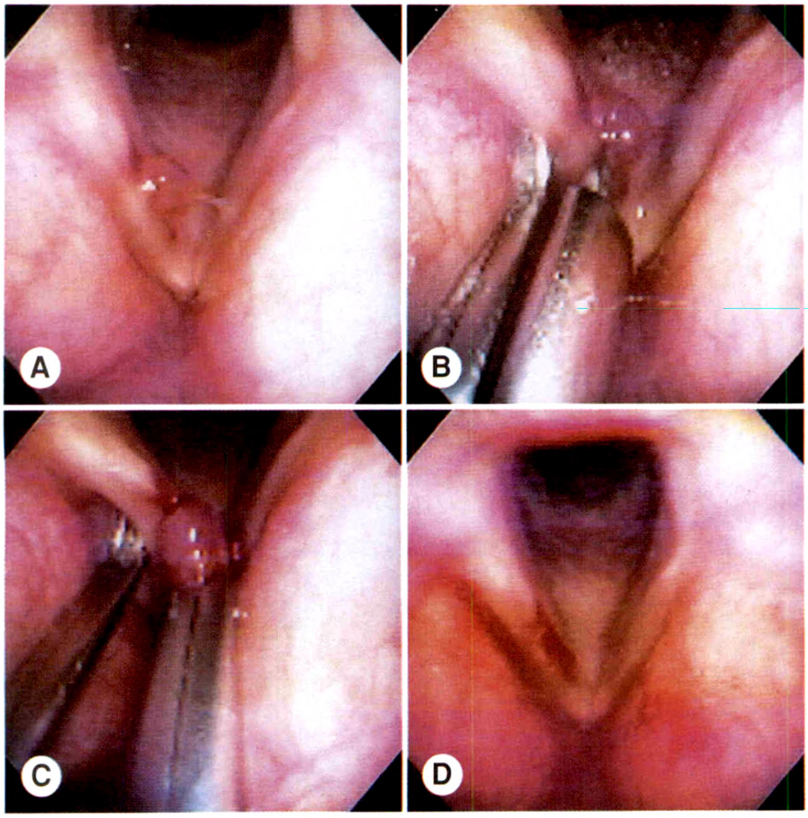

Fig 4. (Case 1) A) Right vocal fold polyp. B) Incision is made with scalpel while vocal fold is retracted by forceps. C) Polyp is grasped with forceps. D) Larynx just after removal of polyp.

tions for 20 cases of vocal fold polyps are summarized in Table 2. Postoperatively, the maximum phonation time was significantly improved (paired *t*-test). The vocal intensity during easy phonation and loud phonation was significantly improved (paired *t*-test). The mean flow rate during easy phonation and loud phonation was significantly reduced (paired *t*-test). In perceptual evaluations, the scores of the voice sam-

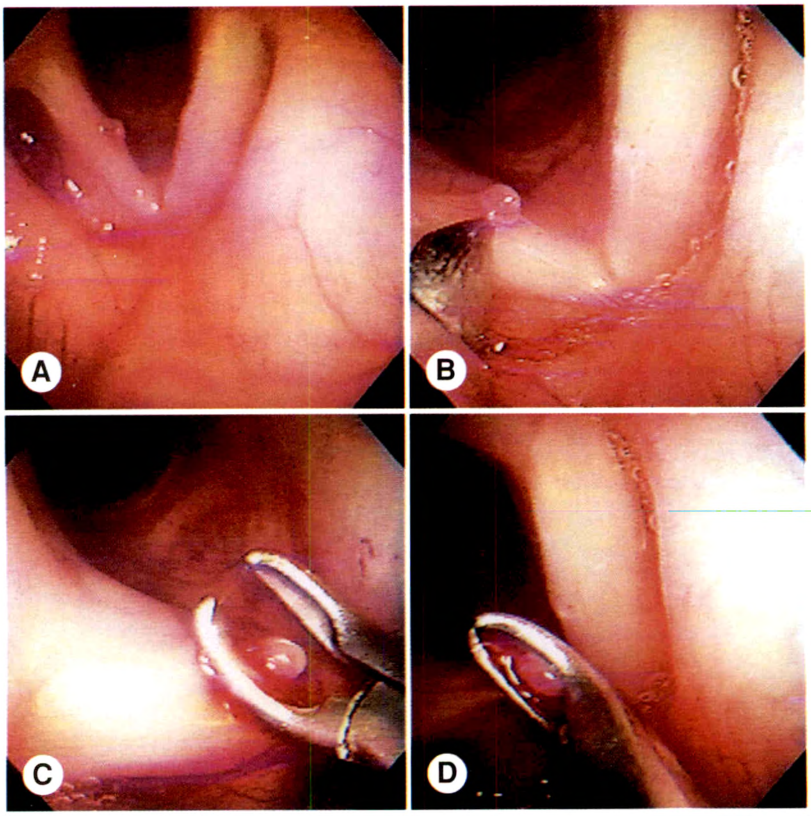

Fig 5. (Case 2) A) Small polyp of right vocal fold. B) Incision is made with scalpel. C,D) Polyp is grasped with forceps.

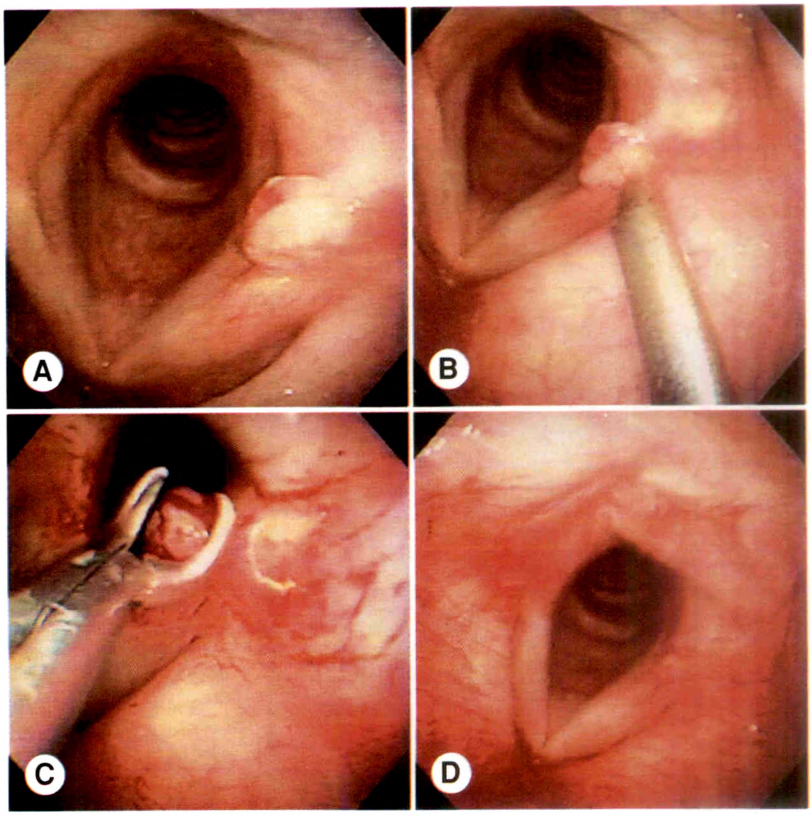

Fig 6. (Case 3) A) Left vocal process granuloma. B) Incision is made with scalpel. C) Granuloma is grasped with forceps. D) Larynx just after removal of granuloma.

ples for roughness and breathiness were significantly improved after the surgery, as shown in Table 3 (Wilcoxon test).

DISCUSSION

Suspension laryngomicrosurgery under general anesthesia with an endotracheal tube has been widely performed. A disadvantage of this method is that the intubation tube obstructs the surgical field. However, the disadvantage has been overcome by the introduction of NLA,[6] in which the patient remains alert without intubation. It allows for vocalization during the course of the operation and facilitates more functional control during the removal of vocal fold swelling.

Later, the application of a stroboscope enabled precise identification of the lesion based on direct observation of mucosal vibration and eventually led Saito et al[4] to devise a novel strategy of laryngomicrosurgery that utilized a stroboflash as a light source under NLA and suspension laryngoscopy. However, NLA has risks, such as suppression of respiration. Vocalization in the presence of a suspension laryngoscope is not always representative of the physiological conditions and may be impossible due to the medication used for NLA. Moreover, suspension laryngoscopy itself is stressful and cannot be carried out in a patient who has difficulty in extending his or her neck. In order to overcome these disadvantages, Saito[3] suggested employment of indirect laryngoscopy under local anesthesia in similar laryngeal stroboscopic surgery to reduce the stress of the patient. However, the method was not widely adopted because of the difficult manipulations required. Since the surgeon had to locate the surgical field using a handheld indirect laryngoscope, the entire operation

TABLE 2. PREOPERATIVE AND POSTOPERATIVE MEAN PHONATORY RESULTS

	Preoperative	*Postoperative*
Maximal phonation time (s)	14.1 ± 5.1	20.4 ± 7.7*
Vocal intensity, easy phonation (dB)	75.4 ± 3.2	77.6 ± 2.8*
Vocal intensity, loud phonation (dB)	83.2 ± 2.8	84.5 ± 3.0*
Mean flow rate, easy phonation (mL/s)	210 ± 79	174 ± 65†
Mean flow rate, loud phonation (mL/s)	294 ± 109	251 ± 114*

Data are mean ± SD.
*Significant improvement (p < .01).
†Significant improvement (p < .05).

TABLE 3. PREOPERATIVE AND POSTOPERATIVE PERCEPTUAL SCORES

	*Roughness Score**				*Breathiness Score**			
	3	*2*	*1*	*0*	*3*	*2*	*1*	*0*
Preoperative (No. of cases)	1	9	10	0	0	1	10	9
Postoperative (No. of cases)	0	0	3	17	0	0	1	19

*Significant improvement (p < .01).

had to be carried out with only 1 hand. Also, the surgical field was difficult to find, and the use of the laryngoscope tended to induce a gag reflex. Mahieu and Dikkers[5] also reported on indirect microlaryngostroboscopic surgery using a rigid telescope and mentioned that it was less suitable for large vocal fold swelling, extensive Reinke's edema, and submucosal swellings, because such lesions required bimanual instrumentation.

A videoendoscope system was developed in which a small CCD chip was built into the tip of the endoscope as an ultraminiature video camera. The images obtained from the target were converted into electronic signals and transmitted to the video system center, and clear dynamic color images were reconstructed and projected onto the color video monitor. Therefore, the problem of obtaining a honeycomb image with a conventional flexible fiberscope is avoided with this system. However, since the tips and flexible portion of the endoscope were thick, the system was used in the gastrointestinal tract and bronchorespiratory tract.[10,11] In 1993, a flexible videoendoscope with a 5-mm–outer diameter tip that allowed transnasal insertion was developed.[9] The EVIS-200 videoendoscope system was used in the present study to provide excellent color reproduction and aid in detection of minute lesions, rather than a conventional fiberoptic endoscope. Videoendoscope-assisted laryngeal surgery presents more accurate diagnosis, more precise manipulation, and better functional control under topical anesthesia.

As a result, good postoperative vocal function was obtained with this technique. In particular, the maximum phonation time was elongated and the voice quality was improved. Scar formation was not observed in any cases. No complications such as bleeding or aspiration of material occurred during the operation. This method has several advantages, as mentioned below. Accurate identification of the lesions permits precise surgical manipulations. The larynx is in the physiological position, because there is no endotracheal tube. Functional monitoring of the patient's voice and vocal fold during phonation indicates the optimum time for termination of the surgery, thus ensuring adequate improvement in the voice quality. Bimanual instrumentation is possible.

Routine use of this method in clinical situations depends on its simplicity and safety, and its being minimally invasive. The level of anesthesia and suppression of the gag reflex was a concern in laryngeal surgery performed under topical anesthesia. A certain degree of gag reflex can be eliminated by using a videoendoscope intranasally, in contrast to an indirect laryngoscope or a rigid telescope intraorally. In all but a few cases, in which the gag reflex was severe, this method could be carried out to completion. However, a few patients became intoxicated, so topical anesthesia should be performed carefully.

A specialized apparatus with a fine tip is indispensable for laryngeal microsurgery in order to maintain the surgical field and make fine manipulations. We devised fine-tipped forceps that allowed manipulation in a plane parallel to the superior surface of the vocal folds without hampering the surgical field, thus helping in the identification of lesions.[7] These forceps were much finer than conventional forceps such as Karl-Reinel laryngeal forceps. A scalpel whose blade can be retracted with a lever for safety during manipulation was devised.

Videoendoscopic laryngeal surgery is most applicable to the removal of vocal fold swellings, such as vocal fold polyps, cysts, nodules, and laryngeal granuloma, but it is also applicable to the biopsy of laryngeal neoplasms, such as epithelial hyperplasia, papilloma, and cancer. Reinke's edema can also be operated on, but this method may be applicable only in those cases in which the lesions are not extensive. Further, this type of phonosurgery is indicated in those cases in which suspension laryngomicrosurgery is difficult or impossible to perform because of anatomic problems such as a short mandible, impaired cervical mobility, and so on.

By videoendoscopic laryngeal surgery, biopsy of the lesion was easily undertaken and sufficient materials for pathological diagnosis were obtained. Suspension laryngomicrosurgery under general anesthesia is too invasive to be used only for biopsy of the laryngeal lesions. In an endoscopic procedure using forceps at the tip of a steel wire inserted into an aspiration channel, the specimen obtained is sometimes insufficient for pathological diagnosis.

In endolaryngeal surgery, the objective of surgical intervention should not be to make the edge of the vocal fold smooth, but to improve the voice quality. In videoendoscope-assisted laryngeal surgery under topical anesthesia, good phonatory results can be obtained by functional monitoring and precise manipulation. This technique is a minimally invasive procedure for resection of benign laryngeal lesions, as well as for biopsy of laryngeal neoplasms.

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CURATIVE RADIOTHERAPY FOR ANTERIOR COMMISSURE LARYNGEAL CARCINOMA

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There is continuing controversy surrounding the most effective treatment of glottic carcinoma involving the anterior commissure (AC). Surgery has been the preferred method of treatment, since studies previously indicated early tumor invasion of the thyroid cartilage at the AC, thereby assuming less curability by radiotherapy (RT). Subsequent laryngeal anatomic studies and refinement of RT techniques have brought into question the ineffectiveness of curative irradiation. A retrospective review of 174 patients with early-stage glottic carcinoma treated with standard fractionation curative RT revealed 34 patients with T1 and T2 lesions involving the AC. Allowing for a follow-up of at least 3 years, we observed only a 12% (4 of 34 patients) local recurrence rate after RT alone, with excellent voice quality and no major complications related to the irradiation. The 4 local recurrences were controlled by total laryngectomy, although 2 patients developed distant metastatic disease. Radiotherapy represents an effective method of treating T1 squamous cell carcinoma of the glottis with AC involvement. The small number of T2 glottic carcinomas in this study prevents a meaningful conclusion concerning treatment of these lesions.

KEY WORDS — anterior commissure, laryngeal carcinoma, radiotherapy.

INTRODUCTION

Squamous cell carcinoma of the laryngeal glottis with normal vocal cord mobility has traditionally been treated with curative radiotherapy (RT) with excellent cure rates (85% to 95%) and preservation of voice quality.[1] Glottic tumors that originate on or extend to the anterior commissure (AC) have been viewed differently. The early literature promoted the concept of early thyroid cartilage invasion by carcinoma involving the AC, rendering these lesions less curable by RT. Various forms of conservation partial laryngeal resection provided oncologically sound excision of the AC and adjacent glottis with voice preservation.[2-5] Refinement of RT techniques, combined with a better understanding of tumor behavior at the AC, has brought into question the ineffectiveness of RT for these lesions. We have had a long-term experience with curative RT treatment of laryngeal glottic carcinomas involving the AC.

METHODS AND MATERIALS

A retrospective chart review was conducted of 174 patients with T1 and T2 squamous cell carcinoma of the glottic larynx treated by our Departments of Otolaryngology and Radiation Oncology between 1972 and 1995. There were 35 patients who had AC involvement by infiltrative carcinoma as documented by direct laryngoscopy and biopsy. Radiographic studies revealed no evidence of gross thyroid cartilage involvement. All but 1 patient had a minimum follow-up of 3 years. All biopsies performed outside the New York University Medical Center were reviewed to confirm the diagnosis. No patient had undergone prior treatment of his or her laryngeal tumor or had evidence of metastatic disease on presentation.

Each chart was reviewed for the gross-appearing nature of the tumor as described at laryngoscopy (ie, exophytic versus infiltrative), the extent of glottic involvement, the presence of subglottic or supraglottic extension, the documentation of vocal cord mobility, the details of irradiation, and the analysis of follow-up. Staging of tumors conformed to the criteria for glottic laryngeal cancer as determined by the American Joint Committee on Cancer (1996).

Curative megavoltage RT technique was used in all patients. In essence, 200 cGy (with ±5% homogeneity throughout the larynx) was delivered once a day, 5 d/wk, from a cobalt-60 machine. All patients

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LONG-TERM TREATMENT RESULTS

T-Stage	*No.*	*NED Locally After RT*	*Neck and Systemic Metastases*	*Primary Recurrence*	*Successful Surgical Salvage*
I	29	27*	0	2	2
II	5	3†	1	2	2

NED — no evidence of disease, RT — radiotherapy.

*One patient with second primary of lung; 2 patients died of unrelated disease at 104 and 126 months.

†One patient with lung metastases 51 months post-RT.

with T1 disease received a total dose between 6,000 and 6,600 cGy (depending on the bulk of residual disease following biopsy) to the larynx via 2 lateral parallel opposed ports encompassing only the larynx. Patients who had T2 lesions received 6,600 cGy to larger fields designed to encompass the entire tumor adequately. What perhaps distinguishes our series from earlier reports of RT is the constant use of cross-sectional anatomy allowing the prescription of RT to an isodose that encompassed all known extent of the tumor (rather than simply prescribing our dose at a point such as midline on the central axis).

RESULTS

Of 34 patients, there were 29 men (ages 37 to 78 years, mean 60 years) and 5 women (ages 46 to 82 years, mean 65 years). Twenty-nine patients had stage T1N0M0 (stage I) glottic cancers with AC involvement. Of 5 patients with stage T2N0M0 (stage II) glottic cancers and AC extension, 1 had supraglottic extension of tumor, 2 had subglottic extension, and 2 had supraglottic extension with impaired vocal cord mobility.

Of the 29 stage I lesions, 13 were exophytic, 14 were infiltrative, and 2 had both exophytic and infiltrative components. Three stage II lesions appeared exophytic, and 2 appeared infiltrative by direct laryngoscopy. All lesions were moderately to well-differentiated squamous cell carcinomas.

Of the 29 stage I lesions, 19 involved the AC and one vocal cord, 9 involved the AC and both vocal cords, and 1 involved only the AC. Of the 5 stage II lesions, 2 involved the AC and one vocal cord, and 3 involved the AC and both cords.

The Table summarizes the long-term treatment results. Of the 29 patients with T1 lesions, 27 (93%) were alive and had no evidence of laryngeal disease at an average follow-up of 80 months (range 22 to 165 months) after curative RT. One patient had tumor recurrence at the AC with extension to the anterior trachea at 37 months following RT, and a salvage total laryngectomy rendered the patient disease-free for the 52 months of follow-up since surgery. One other patient, whose original lesion was confined to the AC, had tumor recur at the primary site 12 months following RT and underwent a partial laryngectomy. This patient then went on to develop a posterior glottic recurrence necessitating a completion laryngectomy a few months later and was rendered free of disease for the ensuing 62 months of follow-up. One patient developed a lung second primary lesion 59 months following RT, but was disease-free in the larynx.

Of 5 patients with T2 lesions, 3 patients had no evidence of disease at the primary site 150 months, 66 months, and 51 months, respectively, following RT. The latter of these patients underwent a partial lobectomy for a lung metastasis 51 months after RT, but was free of disease in the larynx. One patient had a glottic recurrence at 3 months following RT, but was salvaged by total laryngectomy and rendered disease-free for 84 months. The last patient required a total laryngectomy for recurrent laryngeal carcinoma at 48 months following RT, but died of regional and systemic metastatic carcinoma within 12 months.

Primary RT resulted in long-term (≥36 months' follow-up) local control in 30 of 34 patients, or 88% (T1 27/29 or 93%; T2 3/5 or 60%). Four patients ultimately required total laryngectomies and only 1 of these patients died of metastatic disease. No patient died of recurrent local disease. One patient had no evidence of local disease when he died of an unrelated cause 22 months following irradiation.

All patients experienced a transient worsening of voice quality for the duration of RT, with subsequent improvement to a subjectively normal voice. No patients required hospitalization for the completion of RT, nor was there any occurrence of radiation-induced chondritis.

DISCUSSION

Our data demonstrate an 88% initial local control rate of early glottic squamous cell carcinoma involving the AC with curative RT. With surgery reserved for salvage, our ultimate local control rate was 100%. This local control rate compares favorably with those of other reports of AC glottic tumors treated primarily with RT.[6,7] On the other hand, numerous clinicopathologic studies promote conservation laryngeal surgery as the preferred modality for these lesions. This controversy revolves around the anatomic definition of the AC and whether it behaves as a barrier to or a facilitator of the extension of laryngeal carcinoma.

The compartmentalization of the larynx has fascinated anatomists since Hajek[8] injected dye into cadaver larynges and noted abrupt boundaries between the supraglottis, glottis, and subglottis. A well-defined vocal cord submucosal compartment was demonstrated by Reinke.[9] When these boundaries were also found to apply to the lymphatics of the larynx by Pressman et al,[10] it was not long before otolaryngologists began to theorize that intralaryngeal cancer growth might also be similarly restricted. There has been much controversy regarding these restrictions centered around the AC of the glottic larynx.

Kirchner[11] published the first of what would be a 30-year continuum of papers addressing the nature of glottic laryngeal tumor with spread to the AC. He reviewed 13 patients with squamous cell carcinoma of the AC treated by RT and reported a 60% control rate, 8 recurrences, and an overall 25% 5-year recurrence-free rate. He theorized that these poor results were related to the presence of radiation "cold spots," receiving less radiation dosage, with orthovoltage irradiation techniques. Kirchner and Fischer's[12] subsequent study of AC comparing supervoltage RT with surgery demonstrated equally good results with either mode of treatment.

Bagatella and Bignardi[13] further discussed the special characteristics of the AC. They defined a space 1.5 to 2.0 mm high located "between the dihedral angle of the thyroid cartilage and the laryngeal mucosa"[13(p170)] at the level of the AC. This "X-space" is filled with connective tissue and is completely devoid of lymphatics or vessels. They felt that this zone is a barrier to tumor spread from supraglottis to glottis, and that the mucosal appearance overlying this area, just cephalad to the AC, accurately predicts the submucosal extent of tumor: if the mucosa is free of tumor by inspection, there is no submucosal spread or thyroid cartilage invasion.

Yeager and Archer[14] described the first evidence of tumor cells spreading between collagen fibers of the thyroarytenoid tendon, forming tunnels that bored straight through the inner thyroid perichondrium to the thyroid cartilage. The inner thyroid perichondrium is generally considered a strong barrier to tumor spread. By following these collagen fibers as they obliquely pass through the inner thyroid perichondrium to insert on the cartilage, they postulated that tumor cells could bypass this anatomic barrier.

Additional experience and interest led to Kirchner and Carter's[15] publishing a series of whole organ sections of laryngeal carcinoma involving the AC. Studying 412 total or partial laryngectomy specimens, they concluded that 1) tumor is limited by the AC tendon in every case of T1N0M0 squamous cell carcinoma of the glottis, thereby preventing early spread to the thyroid cartilage as long as the tumor is confined to the true vocal cords and AC, and 2) tumor extending above the AC, invading the base of the epiglottis, demonstrates early thyroid cartilage involvement.

In light of these studies, there exist 3 possible scenarios for tumor spread at the AC. First, there may be a glottic carcinoma involving the AC mucosa, and this tumor's spread would be limited by the avascular, lymphatic-poor, dense confluence of tissue formed by the vocal ligament, the thyroepiglottic ligament, the conus elasticus, and the internal perichondrium of the thyroid cartilage.[16] Further, its superior spread into the supraglottis would be restricted by the submucosal "X-space" of Bagatella and Bignardi.[13] These lesions should be highly radiocurable.

Second, deeply infiltrative tumors of the glottis that invade into the thyroarytenoid muscle early might bore into the anterior thyroid cartilage, following slips of collagen fibers of the anterior thyroarytenoid tendon extending beyond the thyroid perichondrium.

Third, tumors that traverse the "X-space" and extend to the base of the epiglottis from the AC demonstrate early epiglottic and thyroid cartilage invasion. Any element of cartilaginous involvement, more prevalent with T2 and T3 lesions of the glottis, would render the tumor more radioresistant.

When T1 AC lesions are segregated from more advanced glottic lesions, a strong argument is made for effective RT,[7,17] especially since surgical salvage results are so encouraging. Studies have documented an 18% to 36% rate of recurrence of AC tumors following curative RT and a surgical salvage of 60% to 100%.[18-21] These statistics are also convincing when T2 lesions are included.[22]

Stevenson et al[23] reported a local control with RT of 100% with T1 lesions involving the AC. The local control of T2 lesions was 75%, with all patients controlled with salvage surgery. Sessions et al[24] had 90% survival of unilateral T1 AC lesions treated with irradiation, which decreased to 50% with involvement of both vocal cords. Hemilaryngectomy resulted in a 74% survival for T1 and T2 lesions treated with primary surgery.

Our study of 34 patients with T1 and T2 glottic carcinomas involving the AC demonstrates control rates of 93% and 60%, respectively, and an overall local control rate of 100% after salvage total laryngectomy. Although voice-sparing conservation laryngeal surgical procedures may be effective in controlling recurrent disease in postirradiated patients,[25] this procedure was not done in our series. Postirradiation recurrent T1 lesions with normal vocal cord mobility and no evidence of thyroid cartilage invasion would certainly be amenable to conservation surgical therapy.

The small number of T2 carcinomas in our series prevents us from conclusively recommending irradiation as the preferred method of treatment. Two patients had restriction in vocal cord mobility that indicated more advanced disease with possible thyroid cartilage extension.

In conclusion, modern RT technique allows for effective curative treatment of T1 carcinomas involving the AC while maintaining excellent voice quality. Salvage surgery provides additional improvement in survival for recurrent tumors.

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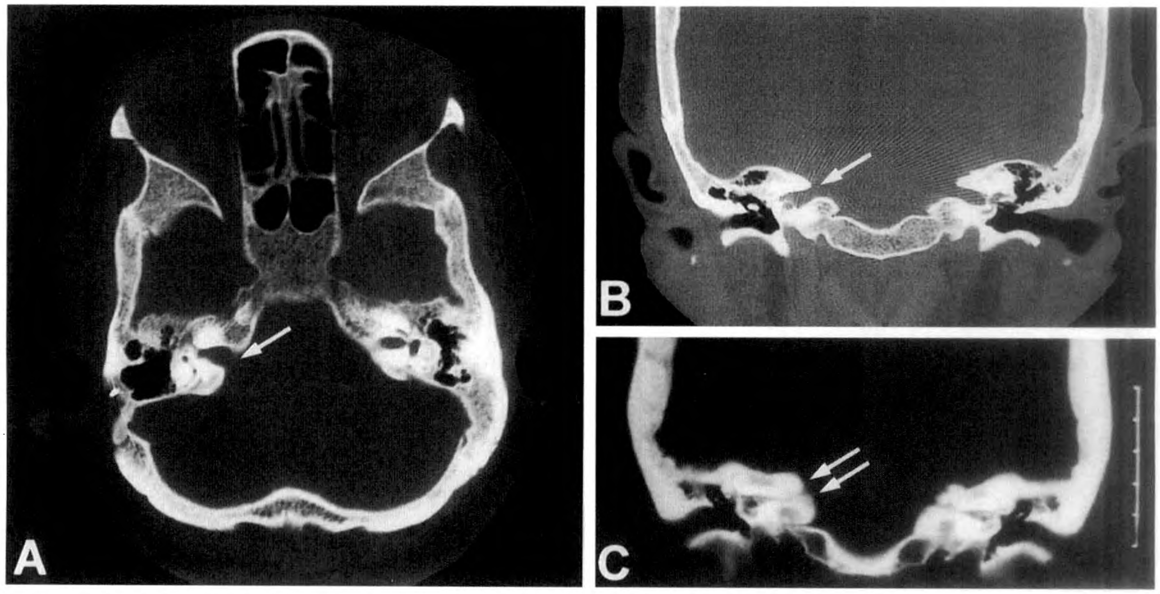

Computed tomographic scans of skull and temporal bones in 2 patients with Camurati-Engelmann disease. A) (Patient 1) Axial view demonstrating patent internal auditory canal (IAC; arrow). Patient is status post cochlear implantation. Note in this view, and in coronal view, thickened bones of cranium and skull base. B) (Patient 1) Coronal view demonstrating patent IAC (arrow). C) (Patient 2) Coronal view. Arrows indicate markedly narrowed IAC. Note thickened bones of cranium.

cies (no response at 110 dB). Type A tympanograms were present bilaterally. Her aided responses were a speech detection threshold of 75 dB hearing level and no detectable speech discrimination ability. On a preoperative computed tomography (CT) scan, the cochlea and middle ears were normal and bilaterally underdeveloped, but aerated mastoids were noted. The CT scans showed widened cortical bone throughout the skull base and cranium, and the IACs were patent (see Figure, A,B). Evaluation by the human genetics department concluded that the patient had Camurati-Engelmann disease.

The patient underwent right-sided cochlear implantation in March 1996 (Nucleus 22, Cochlear Corp, Melbourne, Australia). Postoperatively, she did well, although electrode 5 was deactivated secondary to facial nerve stimulation. On her last audiological evaluation (approximately 1 year postoperative), the patient had a 45-dB speech detection threshold and a 60-dB speech detection threshold on open-set testing. Her open-set speech discrimination ability (W22 4E) was 30% at 75 dB.

Patient 2. A 43-year-old man was referred 1 year after undergoing, at another institution, an attempted left facial nerve decompression for persistent paresis. He suffered iatrogenic loss of the eighth nerve during the procedure and complained of progressively worsening hearing on the contralateral side. Audiological evaluation demonstrated complete deafness on the operated side. On the intact side he had moderately severe hearing loss, and with his hearing aid he had 68% discrimination at 50 dB. A CT scan of the head and temporal bones revealed marked sclerosis and widening of the bones of the skull base. Bilaterally, the IACs were extremely narrowed at the porus acusticus (see Figure, C). Further evaluation, including radiographs of the long bones, confirmed a diagnosis of Camurati-Engelmann disease. The patient was rejected as a potential cochlear implant recipient due to acceptable aided discrimination. Further, should his hearing worsen, he would not be considered a cochlear implant candidate due to the narrowing of the IACs and likely compromise of the eighth nerve. The patient was told that in the future he might be a candidate for an auditory brain stem implant, should the results of ongoing and future Food and Drug Administration trials demonstrate that this auditory prosthesis would be appropriate for him.

DISCUSSION

Cochlear implants have become an effective and common means of restoring hearing in severe-to-profoundly deafened individuals. Absolute and relative contraindications based on age, cause of deafness, anatomy, and concomitant medical conditions have been described.[14] Intuitively, lack of an eighth nerve, or even of a functioning nerve, is also an absolute contraindication. Patient 2 is an example, as the iatrogenic loss of his vestibulocochlear nerve precludes cochlear implantation on that side. Likewise, patients deafened after vestibular schwannoma resection are not candidates for cochlear implantation, if their cochlear nerve has been sacrificed during surgical removal of the tumor.

Congenital absence of a functioning eighth nerve has been reported.[13,15] Rothschild et al[15] have reported 2 children with unilateral stenosis of the IAC with ipsilateral sensorineural deafness. They hypothesized that lack of the eighth nerve fails to induce normal IAC development. Secondary impingement on the nerve by a narrow canal was considered less likely, due to normal facial nerve function. Similarly, Shelton et al[13] reported on failures of cochlear implantation in children with narrow IACs. They concluded that the narrow canal lacked a vestibulocochlear nerve and that such a finding on CT scan in association with sensorineural deafness is a contraindication to cochlear implantation.

Camurati-Engelmann disease can have similar radiographic and clinical findings of narrowed IACs and sensorineural hearing loss. In contrast, however, a previously functional eighth nerve is compressed or vascularly compromised by a progressively narrowing IAC. Stenosis of the IAC is not, however, a consistent or absolute sequela of the disease. Therefore, it is important to assess the entire clinical picture in determining the suitability of the patient for cochlear implantation.

A patient with Camurati-Engelmann disease should be fully evaluated for the cause of the hearing loss. A narrow IAC would heighten one's suspicion of eighth nerve compression, but an intact facial nerve or a lack of vestibular complaints may indicate good cochlear nerve function. In such cases, promontory stimulation may help in determining the potential efficacy of a cochlear implant. Note, however, that the bony changes inherent to Camurati-Engelmann disease may alter the physiological response to stimulation, although this has not been systematically studied. If residual hearing allows, an auditory brain stem response study may also be useful in assessing the function of the eighth nerve.

The age of the patient and the rate of progression of the disease are also factors to consider. An older patient with slowly progressive disease may benefit from a cochlear implant. The degree of IAC involvement should be assessed, and the cause of hearing loss determined. Patient 1 has performed well with her implant and shows no progression toward IAC narrowing or eighth nerve compression. In contrast, a young patient, like patient 2, with rapidly progressive disease and evidence of IAC involvement may already have, or soon develop, retrocochlear deficits. Such a patient may be best served by avoiding surgery or awaiting possible brain stem implantation.

An additional factor to consider is the extent of middle ear involvement. Narrowing of the eustachian tube may lead to chronic otitis media and thus may be a relative contraindication to cochlear implantation. Reports have, however, described successful protocols for implanting patients with histories of chronic serous otitis.[16,17] Further, the bony anatomy of the otic capsule should also be assessed to evaluate the feasibility of placing the electrode array. Protocols have been developed to allow implantation even in the most severe cochlear abnormalities.[11,12]

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EXPERIMENTAL STUDY FOLLOWING INACTIVE IMPLANTATION OF AN AUDITORY BRAIN STEM IMPLANT IN NONHUMAN PRIMATES

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We report changes in the cochlear nuclei (CNs) after 3 months of bilateral auditory deafferentation and simultaneous unilateral implantation of a dummy auditory brain stem implant (ABI) in 6 nonhuman primates *(Macaca fascicularis)*. These specimens were compared to CNs of 9 controls and 7 bilaterally deafferented animals without implantation. The ABI array consists of 3 platinum electrodes mounted on a silicone pad with the back side covered with Dacron. No migration of the ABI was observed. All deafferented animals showed astrocytic reorganization in the CNs. Histologic changes consisted of superficial reactions around the implant, with formation of fibrillar bundles of fusiform cells, and the presence of giant cells close to the Dacron. Other findings were related to surgical trauma. The dummy ABI did not itself provoke serious adverse reactions in the CNs. Our observations support the possibility of ABI reimplantation surgery.

KEY WORDS — cochlear nerve deafferentation, cochlear nucleus, neural prosthesis, neuropathology, nonhuman primate, stereology.

INTRODUCTION

The auditory brain stem implant (ABI) represents a new choice for the treatment of patients with different lesions of the auditory nerve at any level between the spiral ganglion and the cochlear nuclei (CNs) of the brain stem. Clinical observations suggest that prosthetic stimulation of the CN surface is capable of producing auditory sensations.[1-3] However, very few reports have studied the effects of ABI implantation at the level of the CNs. Niparko et al[4] studied the effects of implantation of penetrating electrodes in the CNs after 3 to 4 weeks in a series of 18 guinea pigs, of which 3 animals had the electrodes activated at the time of implantation. Liu et al[5] report the effects of 6 hours of stimulation of the CNs using surface electrodes in a series of 14 guinea pigs.

To our knowledge, there are no reports on primates or humans of the histologic changes at the brain stem after ABI implantation. Likewise, there are no reports on the effects of ABI implantation for a period of 3 or more months. Terr et al[6] describe the morphological characteristics of the tissue sheath around an ABI removed due to infection in the area of the plug, installed on the external surface of the skull in a patient, but no mention is made of the CN or brain stem status.

In order to improve the current ABI design, and to obtain a deeper knowledge of CN anatomy, changes after deafferentation, and effects of ABI implantation, we conducted studies in nonhuman primates. A correct interpretation of the local effects due to the implant, its positioning, and stimuli used are of great interest. The aim of this report is to characterize the effects of a dummy ABI implanted within the lateral recess of the brain stem in the macaque monkey species.

MATERIAL AND METHODS

Animal Model. Twenty-two male cynomolgus macaques *(Macaca fascicularis)* served as subjects for this study. The animals were 3 to 4 years old and averaged 2.6 kg. They were grouped according to the following objectives in the study.

1. Group A (n = 9), control group: study of normal architecture and topographic relations of the CNs. These animals were painlessly sacrificed without any surgical procedure, or after surgery that exposed the cortical surface for an unrelated neuroanatomical tracing study.

2. Group B (n = 7): study of the CNs after 3 months of bilateral translabyrinthine deafferentation.

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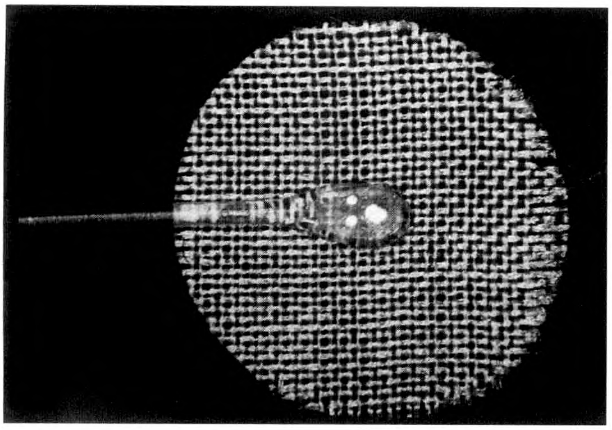

Fig 1. Photograph of dummy auditory brain stem implant (ABI) electrode array with surrounding Dacron mesh (diameter 1 cm). Mesh was cut to fit translabyrinthine approach to brain stem.

3. Group C (n = 6): study of the CNs after 3 months of auditory deafferentation, plus simultaneous unilateral ABI implantation without electrical stimulation.

4. Group D (n = 6): study of the CNs after 3 months of auditory deafferentation, plus simultaneous unilateral ABI implantation with electrical stimulation. Material used was the nonimplanted side.

Housing and Care of Animals. Animals used in this experiment were cared for in compliance with the European Union rules (86/609/CEE). They were housed individually in stainless steel cages (120 × 75 × 100 cm), were fed commercial nonhuman primate chow (B & K Universal), and were supplied

facial nerve and its descending portion, as well as to the cerebellar flocculus that protrudes in the mastoid cavity bracketed by the superior semicircular canal. Opening of the IAC was done proceeding from lateral to medial. The first nerve to be identified and sectioned was the inferior part of the vestibular nerve. The cochlear nerve was carefully exposed and sectioned. A small portion of the nerve was removed to avoid contact with the terminals.

Monkeys of group C had the same surgical procedure to section the cochlear nerve bilaterally and to implant the dummy ABI in the second ear. Following bone drilling, the entire area was inspected. The sigmoid sinus was not completely uncovered, to avoid venous oozing. The flocculus was seen extending superiorly and anteriorly. At times it had to be depressed posteriorly. The dura was opened with a longitudinal incision parallel to the direction of the IAC. The seventh and eighth nerves were inspected and identified by means of anatomic landmarks. The cochlear nerve was sectioned, and the proximal stump was followed to the brain stem. The foramen of Luschka was identified, and any vessel traversing the area was carefully dissected and moved aside. The tela choroidea of the foramen was sectioned transversely behind the eighth nerve in order to obtain access to the lateral recess of the fourth ventricle. The ABI was placed just behind the point of entry of the eighth nerve, in the lateral recess of the fourth ventricle. The electrode array was placed with the active points facing the eighth nerve with a 30°

[redacted]

and 2% DMSO in phosphate buffer until sectioning. The electrodes were left in place, and photographs of the brain stem were obtained for orientation and recording of the electrode position in group C. After this step, the electrodes were removed. Two cases had a different perfusion protocol consisting of a flush with saline and 6 L of 10% formalin, to remain for several months in 10% formalin. Previous to sectioning, these 2 brains were placed in an equilibrating solution as in the previous cases. The brain stem was sectioned at the level of the cerebral peduncles and placed in a freezing microtome, such that sections orthogonal to the main axis of the brain stem were obtained. Thirty-micron serial sections were prepared in all cases, with an interval of 240 μm between adjacent sections. Every eighth series obtained was stained with thionin for cytoarchitectonic and damage evaluation purposes. The remainder were stored in a cryoprotecting solution until use. One additional series, spaced 480 μm from the first section, was immunostained with a monoclonal antibody (Sigma, 1: 200) for the demonstration of glial fibrillary acidic protein (GFAP). These sections were reacted with the avidin-biotin complex (ABC Biostar, Biomeda) with diaminobenzidine and hydrogen peroxide as chromogen. The reaction was further intensified with osmium and thiocarbohydrazide.

Stereology. The purpose of the analysis was to estimate the CN volume with an unbiased method and to investigate the differences between the CN volumes of control, deafferented, and implanted animals.

Both CNs of the 9 animals from (control) group A were analyzed. Both CNs of the 7 animals from (deafferented CN) group B and the 6 nonimplanted CNs of animals from group D were analyzed. Only 4 CNs from (implanted) group C animals were selected. Two animals were excluded because of severe surgery-related damage to the CNs (see Results), to avoid a selection bias in the stereological analysis.

Thionin-stained sections (Nissl stain) were selected for volume measurements. We used Cavalieri's principle,[7,8] which requires systematic section of the whole CN and a random selection of the sections. A mean of 10 sections for each side of the CNs was studied.

The workstation for stereology consisted of a Leitz microscope, a motorized stage for automatic sampling, a personal computer with the software Olympus Denmark CAST-Grid system installed, and a video camera. A grid of crosses is superimposed onto the video image of the section for Cavalieri's analysis. The distance between the crosses is variable regarding the size of the object to be measured. In every section studied, occurrences of the right superior angle of the crosses on the CNs were counted. The following formula was applied to the data obtained: Total Volume = $\sum P(a/p)T$, where $\sum P$ is the summation of all the crosses counted in the different sections, which should be around 100 crosses. T is the mean distance between the selected sections, in our study a value of 240 μm. And (a/p) is the constant of the grid, in our study a value of 288,124.78 μm^2. The estimation of the sampling error was calculated according to Gundersen and Jensen's[7] formula.

Statistical Analysis. The estimated CN volumes from the 3 groups were compared by a nonparametric test (Kruskal-Wallis). The Mann-Whitney test was used to compare the CN volumes between 2 groups. Statistical analysis was performed with the package SPSS for Windows.

RESULTS

Tolerance of Surgical Procedure. All monkeys tolerated the surgical procedure well. No cases of mortality, meningitis, intracranial complications, or facial palsy were observed. Three monkeys developed minor complications related to the surgical wound. During the 3 months of survival, there were no substantial differences in behavior or clinical status of monkeys from group B versus group C.

Macroscopic Postmortem Analysis. During the autopsy, no relevant anatomic alterations were observed in the brains of the 8 control monkeys from group A. In all monkeys from group B, bilateral section of the cochlear nerve was confirmed, showing no macroscopic damage of the cerebellum or the brain stem.

Similarly, in group C, bilateral section of the cochlear nerve was confirmed. The electrode positioning was studied in detail to evaluate possible migration of the ABI, which was not observed in any of the animals. All the ears implanted showed new bone formation of the mastoid cortex and fibrosis around the cable. The bone wax used to close the translabyrinthine approach was in place around the silicone cable, showing no macroscopic signs of intolerance or inflammation. Intracranially, the silicone cable was wrapped by fibrous tissue (Fig 2) with a thickness of 2.331 to 2.664 mm. This capsule had to be opened longitudinally with a No. 11 scalpel to enter the lateral recess. At this level, a slight fibrous reaction was observed around the electrode array. In all animals, the side of the electrodes was easily detached from the brain stem, without observable signs of scarring, under the surgical microscope. The other side (covered with Dacron mesh to stabilize the ABI) appeared covered with pia mater in 4 monkeys (C2, C3, C4, and C6), thus fixing the ABI to the cerebellar aspect of the lateral recess. In these cases, it was necessary

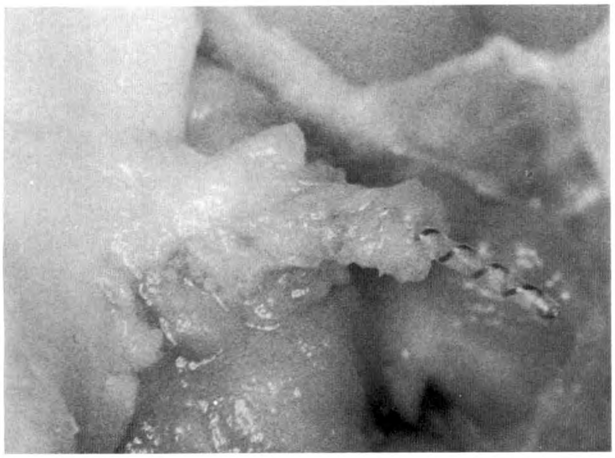

Fig 2. (Case C5) Photograph of specimen in autopsy. Dummy ABI is seen in place within lateral recess of fourth ventricle. Notice scar tissue surrounding cable.

to section the Dacron mesh from the ABI array and leave the mesh in situ. This pia mater reaction was not observed in monkeys C1 or C5, and in these cases the whole ABI array was removed without difficulty.

In the first 4 cases (C1 to C4), the electrode array was implanted medial to the CNs, too deep into the lateral recess. In C5 and C6, the ABI was correctly placed, at the level of the CNs. In 3 cases (C1, C2, and C6), the cerebellar flocculus behind the ABI was reduced in size and presented a yellowish to brownish color.

Once the ABI array was removed, examination of the surface electrodes showed the integrity of the array in all cases except C1, in which an electrode was missing.

Histologic Analysis. The histologic examination of the nuclei showed striking differences between controls and deafferented animals. Although all portions of the CNs (anteroventral, posteroventral, and dorsal) could be readily identified, they were remarkably shrunken after bilateral section of the cochlear nerves. Deafferented cases had normal appearance of neurons, but the density of glial processes appeared greater in all portions of the CNs. In addition, the entrance of the nerve presented a vacuolated appearance and an overall disorganization of the normal astrocytic processes pattern, particularly evident in the GFAP-stained series.

The cases in which an ABI was placed showed variability according to the outcome of the surgery. Cases C1 and C2 presented an almost complete destruction of the CNs, due to direct damage during placement of the ABI because it was placed intraparenchymatously and not on the CN surface (Fig 3A).

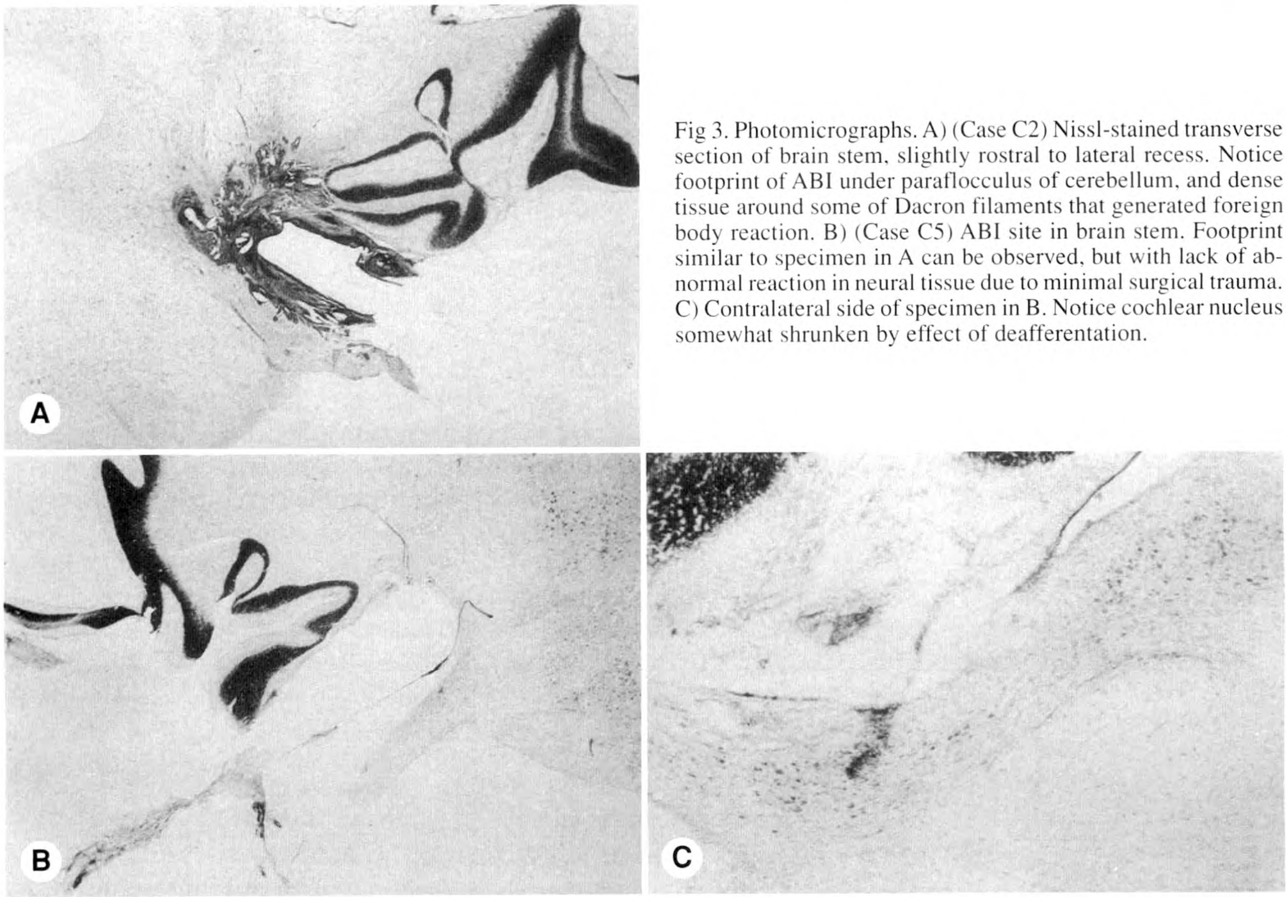

Fig 3. Photomicrographs. A) (Case C2) Nissl-stained transverse section of brain stem, slightly rostral to lateral recess. Notice footprint of ABI under paraflocculus of cerebellum, and dense tissue around some of Dacron filaments that generated foreign body reaction. B) (Case C5) ABI site in brain stem. Footprint similar to specimen in A can be observed, but with lack of abnormal reaction in neural tissue due to minimal surgical trauma. C) Contralateral side of specimen in B. Notice cochlear nucleus somewhat shrunken by effect of deafferentation.

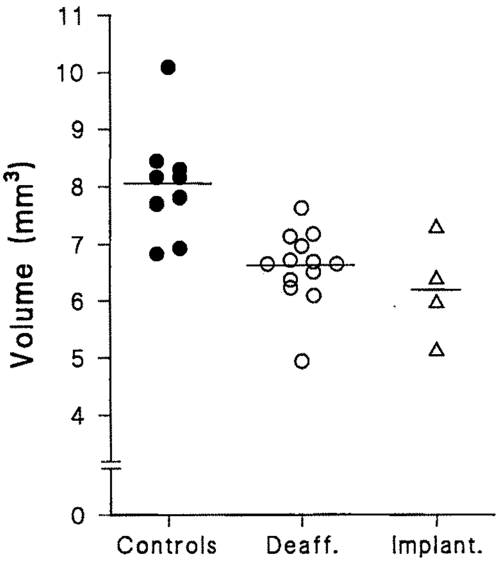

Fig 4. Scattergram representing estimated volume of cochlear nuclei (CNs) in control, deafferented, and implanted animals (grouped as described in Material and Methods). Bar equals mean value of estimated volume.

The surrounding pia mater showed a thickening consisting of a fusocellular reaction organized in fibrillar bundles and a few lymphocytic cells. Neither leukocytic infiltration nor other signs of acute inflammation were observed. In the vicinity of the destruction, some hemosiderin was evident, and inflammatory cells were present around vascular structures in adjacent cerebral tissue. Case C3 had less destruction of the CNs, but the ABI penetrated deeply into the brain stem, up to the inferior cerebellar peduncle. Glial and foreign body reactions replaced the cerebral parenchyma. Some Dacron filaments, clearly demonstrated by their refringence, were included in this reaction. Similar changes were observed in the rest of the CNs in case C2. Cases C4, C5, and C6 had much less damage, so that the CNs were fairly intact and showed a histologic pattern similar to that of group B. In these cases, the ABI was successfully placed at the level of the lateral recess of the fourth ventricle, and only a minor pial thickening was observed. In case C5, a deep indentation in the surface of the brain stem at the level of the CNs was observed, but the cerebral tissue was intact (Fig 3B,C). There was no evidence of vascular compression.

Stereology. Volumes were determined for the control series and compared to the deafferented series with and without ABIs. The values obtained for each group are shown in Fig 4. The mean estimated volume of the CNs was 8.05 mm^3 in control animals, 6.60 mm^3 in deafferented animals, and 6.18 mm^3 in the 4 implanted animals studied. The coefficient of error due to the method was 3.78%.

The statistical analysis of the estimated CN volume with the Kruskal-Wallis test revealed significant differences ($p = .0013$, $p < .005$) between the 3 groups. The CN volume was compared between every 2 groups with the Mann-Whitney test. The results of this test show that

1. There are significant differences between the CN volumes of animals from the control group (A) and the deafferented group (B; $p = .0007$).

2. There are significant differences between the CN volumes of animals from the control group (A) and the implanted group (C; $p = .0134$).

3. There are no significant differences in CN volume between deafferented and implanted animals (groups B and C; $p = .3352$).

DISCUSSION

In contrast to the results of Niparko et al,[4] our animal model permitted a translabyrinthine approach to the brain stem, similar to the approach used in humans for ABI implantation. This surgical approach provides an optimum angle of vision to the lateral recess and the CNs.[1] The animals tolerated satisfactorily the anesthetic and surgical procedures, developing no short-term or long-term major complications. Therefore, we think that this species offers optimal characteristics for studies of chronic implantation.

According to the surgical result, we observed 2 locations of the ABI within group C:

1. Intraparenchymatous location. In these cases the position of the electrode array was too deep and there was total destruction of the CNs in primates C1 and C2 and partial destruction in C3 and C4. The lesion was caused during surgery, by mechanical disruption of the lateral recess. In these animals, apart from the surgical CN lesion, there is also an intraparenchymatous reparative reaction, and a foreign body reaction against the array.

2. Extraparenchymatous location. In primates C5 and C6 the ABI was placed on the surface of the brain stem, over the CNs. There was no disruption of the lateral recess and no neural tissue damage, and thus the CNs were similar in cellular appearance to group B specimens. There was only a leptomeningeal reaction around the Dacron filaments that facilitated the stabilization of the ABI.

Our surgical experience agrees with Brackmann et al,[1] Terr et al,[9] and Monsell et al[10] that the best anatomic landmarks for locating the lateral recess and CNs are the roots of the seventh, eighth, and ninth cranial nerves and the choroid plexus. Nevertheless, the histologic analysis reveals that the position of the electrode array was too deep in the lateral recess in the first 4 cases. The excessive depth of the implant, associated with the surgical maneuvers used to reach the lateral recess, was probably responsible for the histologic damage in the CNs and surrounding structures. Therefore, it is mandatory to perform a delicate surgical procedure around the lateral recess and on the surface of the brain stem. We should avoid aggressive maneuvers for the dilation of the foramen of Luschka, an inappropriately directed insertion, too many repositioning movements of the ABI, and the use of inadequate surgical instruments that may cause intraparenchymatous lesions of the brain stem. The reduced size of the CNs and their superficial situation in the brain stem indicates that even minimal trauma causes neural loss, reducing the ABI-stimulable neural tissue.

The ABI implantation was performed in the first 4 cases following the indications proposed by Brackmann et al[1] and Shannon et al[2] to obtain auditory stimulation without side effects. Nevertheless, the histologic study of our cases revealed lesions caused by an excessive penetration of the implant because of the disruption of the lateral recess and the intraparenchymatous location of the ABI. The explanation could be that in *M fascicularis* the width of the lateral recess is under 3 mm, while in humans it is 7 mm,[10] and the recess is also located more laterally in monkeys than in humans. In monkeys C5 and C6, implantation was performed more laterally. The distal half of the electrode array was inserted, leaving the proximal half outside the recess, over the stump of the eighth cranial nerve. This led to a substantial decrease in tissue damage and better positioning of the electrodes with regard to the CNs.

Our results indicate that direct damage by the ABI to the brain stem is minimal if the surgical procedure is carefully undertaken. In such cases we have not observed relevant differences in the CNs in monkeys from groups B and C, although the number of specimens with an adequate ABI positioning is reduced. The volume of the CNs of the primates from the control group (A) was significantly higher than the volume of deafferented (B) or implanted animals (C), but there are no significant differences among animals from groups B and C.

Preliminary results of stereological studies with 2 animals from each group have not shown significant differences in the number of neurons of control, deafferented, or implanted CNs. Therefore, the decrease of volume should not be attributed to a loss of neurons within the CNs. This is in accordance with other studies[11-13] performed in adult animals that did not show a significant loss of neurons in the CNs after auditory deafferentation.

The decrease of volume of deafferented CNs should not be attributed, either, to changes in the astrocyte population. Our stereological studies[14] of the glia in control, deafferented, and implanted CNs do not support this contention, because no significant differences in the total length or density of glial processes could be demonstrated.

On the basis of our results and according to other authors,[11-13] the decrease of volume in deafferented CNs is most likely due to the loss of afferents from the cochlea, and possibly due to a shrinkage of neuronal cytoplasm.

The platinum electrodes and the silicone were in direct contact with the CN surface and were well tolerated. We did not find lesions within the nuclei in the macroscopic or microscopic examination that could be attributed to these materials. The presence of hemosiderin in the vicinity of the destruction areas of primates C1 and C2 reflects a quite traumatic implantation. As stated previously, if the ABI implantation is atraumatic, only a minor pial thickening is observed in the brain stem, and no foreign body reaction is present. On the other hand, the Dacron mesh produced a desired fibrotic reaction, sometimes with the presence of multinucleated giant cells, that contributed to the stabilization of the array. In conclusion, we think that these materials are suitable for implantation on the CNs of the brain stem, and have an adequate biotolerance for the avoidance of histologic lesions in implanted primates.

Migration of the electrode array was not observed in any of the implanted monkeys, so that records of the site of implantation coincided with the site found at autopsy. This is in accordance with results observed by Shannon et al[2] in 17 patients who received an ABI. They suggest that the close fit of the electrode array into the lateral recess holds the electrode assembly in position during the critical days immediately after implantation while fibrous tissue is invading the Dacron carrier, fixing the electrode in place.

One of the questions concerning any implantable device is the possibility of necessary removal and subsequent reimplantation. Our autopsy findings show that the cable is wrapped by fibrous tissue that can be removed easily without brain damage. This is in accordance with Terr et al,[6] who described a case of an electroauditory prosthesis implanted in a human that was removed due to infection in the area of the electrical plug on the external surface of the skull. Nevertheless, there are no previous reports about the effects of electrode array removal within the lateral recess. The side of the array containing the electrodes can be easily detached, and we have not observed damage histologically in the brain stem. The side covered by Dacron mesh was firmly adhered in 4 of the 6 cases, so that it was necessary to detach and leave the Dacron mesh in place. This procedure was not difficult from the surgical point of view. We think that the reaction to the Dacron does not represent a contraindication or make an ABI reimplantation procedure more difficult.

CONCLUSIONS

The destruction of the CNs and the intraparenchymatous reaction observed in the first 4 cases should be attributed to surgical damage, and not to any reaction to the ABI materials. It is crucial to have a good command of the CN topography to implant the ABI properly. Otherwise, there exists the possibility of injuring the CNs and surrounding structures as well as failing to get proper stimulation. As in cochlear implant surgery, it is necessary to perform "soft surgery" to implant an ABI.

The presence of a dummy ABI did not itself provoke important adverse reactions on the surface of the CNs, though some specimens did not have a proper implantation. The thin capsule observed around the cable and the electrode array and the absence of injury on the CNs after autopsy explantation support the possibility of reimplantation surgery.

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PRODUCTION OF DIAGNOSTIC RULES FROM A NEUROTOLOGIC DATABASE WITH DECISION TREES

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A decision tree is an artificial intelligence program that is adaptive and is closely related to a neural network, but can handle missing or nondecisive data in decision-making. Data on patients with Meniere's disease, vestibular schwannoma, traumatic vertigo, sudden deafness, benign paroxysmal positional vertigo, and vestibular neuritis were retrieved from the database of the otoneurologic expert system ONE for the development and testing of the accuracy of decision trees in the diagnostic workup. Decision trees were constructed separately for each disease. The accuracies of the best decision trees were 94%, 95%, 99%, 99%, 100%, and 100% for the respective diseases. The most important questions concerned the presence of vertigo, hearing loss, and tinnitus; duration of vertigo; frequency of vertigo attacks; severity of rotational vertigo; onset and type of hearing loss; and occurrence of head injury in relation to the timing of onset of vertigo. Meniere's disease was the most difficult to classify correctly. The validity and structure of the decision trees are easily comprehended and can be used outside the expert system.

KEY WORDS — artificial intelligence, decision trees, diagnostic process, dizziness.

INTRODUCTION

Medical decision-making can be thought to be classification: a physician classifies a patient, on the basis of his or her knowledge and experience, to a certain disease group. Medical databases contain many data from which programs using artificial intelligence can extract general disease classification models. These models can be used to aid diagnostic decision-making and to transfer diagnostic knowledge from experts to inexperienced young physicians. In the decision program using artificial intelligence in adaptive, ie, self, learning, a new knowledge of the disease can be achieved.

Top-down induction of decision tree (TDIDT) methods are machine learning methods that represent classification models in the form of decision trees.[1] A decision tree is a recursive knowledge structure comprising nodes and leaves (Fig 1). The inner nodes represent tests based on features of example cases, and the leaves represent classes, ie, decisions. Because of their symbolic and, thus, intelligible representation, decision tree methods are well suited for medical decision-making, in which explanations of decisions are also needed.

Decision tree methods have been applied in various problem domains.[2] In medicine, they have been utilized in the diagnosis of thyroid-related disorders[3,4] and to investigate the relationship between clinical and neurophysiological assessments of spasticity,[5] for instance. In this study, we have used Quinlan's See5 classification system,[6] a descendant of C4.5,[7] to acquire diagnostic knowledge for the 6 otologic diseases involving vertigo.[8]

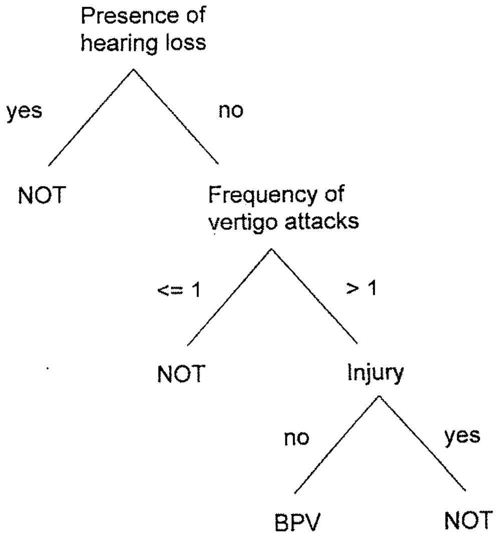

Fig 1. Decision tree for benign positional vertigo. Tree was constructed from all patient cases by using 53 attributes and continuous coding for ordered discrete attributes. BPV leaf represents decision "benign positional vertigo," and NOT leaves represent decision "not benign positional vertigo."

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MATERIALS AND METHODS

Patients. The information on the patients in the database was collected from the vestibular unit of the Helsinki University Hospital. The patients referred to the vestibular laboratory filled out a questionnaire concerning their symptoms, earlier diseases, accidents, and use of tobacco and alcohol.[8] The information was stored in the expert system ONE.[9] From the database of ONE, we selected the 6 largest patient groups, altogether 564 patients, with vertigo. The diseases were Meniere's disease, vestibular schwannoma, vestibular neuritis, benign positional vertigo, traumatic vertigo, and sudden deafness. The diagnoses were confirmed by an experienced specialist in the field of otoneurology.

Attributes. The database of ONE was collected with the aid of 170 questions.[10] The information in the database can be divided into the following classes: 1) symptoms, (eg, vertigo, hearing loss, tinnitus, unsteadiness, headache, anxiety, and neurologic symptoms); 2) medical history, including the use of ototoxic drugs, head or ear trauma, noise injury, ear infections and operations, specific infections (borreliosis, chlamydia, lues), and other diseases; and 3) findings from such examinations as clinical and neurotologic examinations (saccadic and pursuit eye movements, posturography, electronystagmography, brain stem audiometry, and electrocochleography), audiometry, imaging (computed tomography [CT], magnetic resonance imaging [MRI]), and fistula tests.

Case history data have proven to be the most valuable in classifying the 6 otologic diseases involving vertigo.[8] With this patient information, we built decision trees stepwise, using increasing numbers of attributes. First we built the decision tree with the 5 earlier-defined key questions containing only 5 attributes.[8] At the second stage, we added up to 38 parameters. These attributes gave good prediction accuracy with genetic algorithms and contained only a little missing information. Then we added attributes that we thought were valuable in the classification, to 53. We further increased the number of attributes in 2 stages, first to 110 and then to 123, to see if additional information was beneficial for the diagnostic work. The more attributes we had, the more there was missing information. The stepwise increase and the attributes are presented in Table 1.

Decision Tree Process. The See5 classification system takes a set of example cases described by a fixed collection of attributes, ie, features. Each case belongs to a known class. The See5 examines cases and selects the attribute that best discriminates between the classes. This attribute becomes the root of the decision tree and splits example cases into sub-

TABLE 1. STEPWISE ADDITION OF ATTRIBUTES IN LEARNING PROCESS

First 5 attributes
  Time from onset of symptoms*
  Frequency of vertigo attacks*
  Duration of a vertigo attack*
  Duration of hearing loss
  Head injury*

38 Attributes
  First 5
  Age at first symptoms*
  Intensity of vertigo attack*
  Score for rotational vertigo*
  Score for floating sensation*
  Score for position-induced vertigo*
  Unsteadiness
  Falls*
  Type of hearing loss*
  Location of hearing loss (2)
  Fluctuation in hearing*
  Duration of tinnitus*
  Strength of tinnitus*
  Strength of nausea*
  Lightheadedness and other functional symptoms*
  Ear illness
  Ear operation*
  Ear trauma (2)*
  Noise exposure*
  Spontaneous nystagmus*
  Electronystagmography*
    Spontaneous nystagmus
    Caloric asymmetry
    Response with 44°C right
    Response with 44°C left
  Posturography*
    Eyes open
    Eyes closed
  Pursuit eye movements
    Gain amplitude
    Gain latency
  Tone burst audiometry at 500 Hz and 2 kHz*

53 Attributes
  Earlier 31 (marked with *)
  Vertigo
  Hearing loss
  Movement difficulties
  Tinnitus
  Headache
  Score for visually induced vertigo
  Score for pressure-induced vertigo
  Score for physical activity–induced vertigo
  Anxiety
  Score for functional symptoms
  Hearing loss during vertigo attack
  Location of tinnitus
  Central nervous system symptoms
  Syncope
  Visual blurring
  History of ear disease
  History of ear perforation
  History of ear discharge
  Past cholesteatoma
  Head-shaking nystagmus
  Finger-nose test
  Diadochokinesis

110 Attributes
  All earlier 60
  Headache during vertigo attack
  Headache
  Migraine
  Unsteadiness outside vertigo attacks
  Dysarthria
  Cranial nerve palsy
  Paresthesia in face
  Use of ototoxic or vestibulotoxic drugs, including painkillers, antidepressives, diazepam (6)
  Use of alcohol or tobacco (2)
  Specific questions on ear illness and operations (8)
  Other diseases (11)
  Results of saccadic eye movements (6)
  Detailed questions on head injury (4)
  Result of computed tomography
  Tone burst audiometry at 1 and 4 kHz
  Age group

123 Attributes
  Earlier 110
  Tone burst audiometry at 8 kHz
  Result of speech discrimination test
  Query if specific examinations were done
  Use of diuretics, aminoglycosides, barbiturates, chlorpromazine, cytostatics

Numbers in parentheses are numbers of questions.

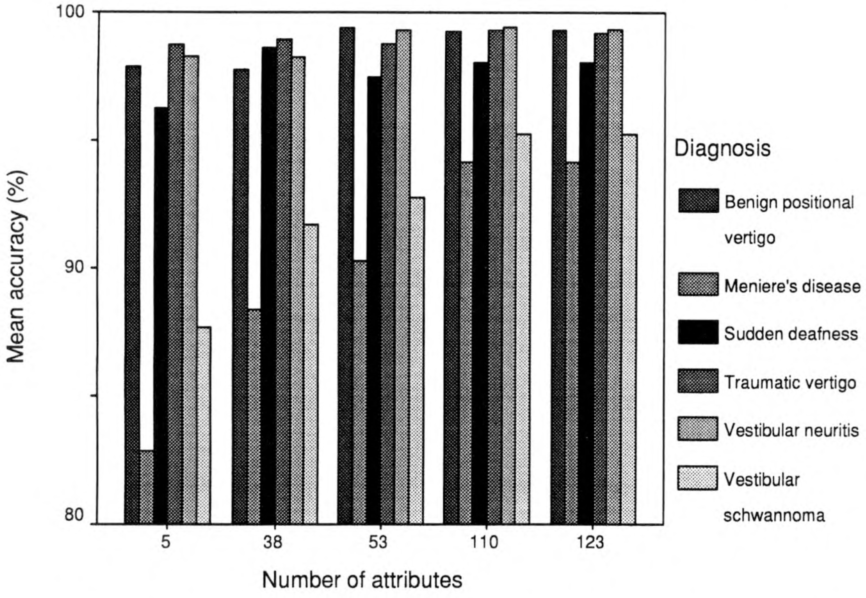

Fig 2. Mean prediction accuracies for decision trees constructed in 10 times–repeated 10-fold cross-validations. Ordered discrete attributes were coded as continuous.

sets. For each subset, a decision tree is generated in the same manner. This recursive process continues until each subset adequately represents a single decision class.

In real-world classification, tasks, data are typically noisy. Noise results in overly complex, incomprehensible decision trees. To produce more comprehensible decision trees, the See5 uses heuristic pruning methods. These methods simplify a decision tree by deleting those parts of a tree that do not have a significant role in the classification of unseen cases. Another problem with real-world data is incompleteness. To cope with missing attribute values, See5 uses a probabilistic approach.

For each diagnosis, several classification models were generated using variable numbers of attributes. The numbers of attributes we used were 5, 38, 53, 110, and 123. We were interested in whether the type of attribute has an effect on learning results, so we coded the ordered discrete parameters as both discrete and continuous. First, we built 10 decision trees for each disease using all the patient cases. The training examples were given in the form of class versus other classes. Because data were quite scarce, we did not use a separate test set, but a method called N-fold cross-validation,[7,11] to estimate the classification accuracy of the decision trees. In the N-fold cross-validation, data are randomly divided into N nearly equal-sized subsets with the same class distribution. For each subset, a decision tree is constructed from N – 1 subsets and tested by the Nth subset. We repeated 10-fold cross-validation 10 times for each classification model to obtain a reliable estimate of prediction accuracy.

RESULTS

The decision trees were constructed independently for each disease (Meniere's disease, vestibular schwannoma, vestibular neuritis, benign positional vertigo, traumatic vertigo, and sudden deafness). All the solved patient cases were used in the construction of the decision trees. Then, the prediction accuracies of the decision trees were estimated with the 10 times–repeated 10-fold cross-validation. The accuracy of decision trees with different numbers of attributes is presented in Fig 2, and the accuracy and true- and false-positive rates for the best decision trees are

TABLE 2. RESULTS FROM 10 TIMES–REPEATED 10-FOLD CROSS-VALIDATIONS FOR BEST DECISION TREES

		True-				*False-*	*Discrete*
	True	*Positive*	*False*	*True*	*False*	*Positive*	*Ordered*

[redacted]

shown in Table 2.

The prediction accuracy was calculated as a ratio of correctly classified cases to all cases: Accuracy = Pt + Nt/PT + NT, where Pt is true-positive cases, Nt is true-negative cases, PT is the total number of positive cases, and NT is the total number of negative cases. Performance of decision trees was also described by true-positive rate and false-positive rate: true-positive rate = Pt/PT, and false-positive rate = Pf/NT, where Pf is false-positive cases. The true-positive rate shows how well the disease cases in question were found from all patient cases. The false-positive rate shows how many patients from other disease groups were classified as belonging to the group in question (false-positive cases). Fifty-three questions proved to be sufficient for good diagnostic accuracy. In all diseases except sudden deafness, the addition of attributes increased the diagnostic accuracy.

Vestibular Schwannoma. For vestibular schwannoma, the estimated prediction accuracy was 95%. This was a high accuracy percentage and can be explained by the high number (n = 110) of attributes used in the rule processing. Both the data from CT or MRI and the results of neurotologic tests were used in the classification. Just 38 attributes leads to a true-positive rate of 80% (distinguishing vestibular schwannoma cases from other diseases), but it required 110 attributes to build a decision tree that excluded the false-negative cases. The trees that classified vestibular schwannoma had the question of the presence of vertigo at the root of 6 of the 10 trees. Only half of the patients with vestibular schwannoma had had vertigo (n = 65, 51%). The other root attributes were duration of vertigo (3/10) and high score for rotational vertigo (1/10). The root attribute is thought to be the most important one in the classification.

Other attributes meaningful in the trees concerned the caloric asymmetry in electronystagmography, deterioration of hearing, sudden slips, and the results of imaging studies. The absence of nausea or only slight nausea, commonly seen in patients with vestibular schwannoma, was included in 3 of the trees.

The patient cases that the best decision tree, constructed from all cases, could not classify correctly had a large tumor with accompanying symptoms. The false-positive cases belonged to the sudden deafness group.

Benign Positional Vertigo. Benign positional vertigo was estimated to be classified correctly in 99% of the cases. Both the true-positive rate (98%) and the false-positive rate (1%) were good. There was 1 case of benign positional vertigo that the decision tree did not find, due to lacking information on the case history. The 3 false-positive cases had missing information on critical attributes (duration of vertigo attack, score for position-induced vertigo) that interfered with the reasoning. Two of these had vestibular schwannoma, which could not be excluded, since the decision tree had only 53 attributes and the results of imaging studies was not among those.

The high accuracy in detecting benign positional vertigo was reached with few questions. In the root of the decision tree, there was always a parameter confirming the absence of deviant hearing. Other attributes concerned duration and occurrence of the vertigo attack, absence of head trauma, and lack of symptoms from the central nervous system (visual blurring, double vision, and paresthesia).

Meniere's Disease. The estimated accuracy in identifying Meniere's disease was the lowest, being 94%. With 110 attributes, we reached a good true-positive rate of 99%, but with these rules 9% (n = 30) of the other disease cases were classified as belonging to Meniere's disease. One of the Meniere's disease patients that the decision tree did not find had severe bilateral Meniere's disease, and the other had only mild short-lasting vertigo. The false-positive cases all had the full triad of vertigo, hearing loss, and tinnitus, but they either had confirmed vestibular schwannoma or sudden deafness with accompanying vertigo.

The root attribute in the decision tree was the occurrence of hearing loss in 8 of the 10 trees and the occurrence of tinnitus in 2 of the 10 trees. Other attributes included the intensity of tinnitus, the duration and occurrence of vertigo attacks, the occurrence of head or ear injury, and a rather high score for nausea.

Sudden Deafness. The estimated accuracy in diagnosing sudden deafness was 99%. With sudden deafness, the highest true-positive rate was reached with only 38 attributes. The augmenting of attributes slightly diminished the true-positive rate, partly because of the enlarged number of confounding factors, which made the case resemble vestibular schwannoma or Meniere's disease, and partly because of increasing the amount of missing information. With 38 attributes, the first 3 attributes in the decision trees remained the same in 88 of the 100 trees constructed in the cross-validation tests. The 3 attributes were the duration of hearing loss, the type of hearing loss, and the question concerning fluctuation of hearing. It appeared that the combination of these 3 attributes was optimal in diagnosing sudden deafness. With 53, 110, and 123 attributes, the structure of the decision trees varied more. The false-positive rate was low in each attribute group.

Five of the 10 decision trees for sudden deafness contained only the root node labeled NOT, which means that every case to be classified with these trees will be classified as not having sudden deafness. These decision trees were built with 5 attributes (discrete ordered attributes were coded as both discrete and continuous) and 38, 110, and 123 attributes (discrete ordered attributes were coded as discrete). The root attribute in the other 5 decision trees for sudden deafness was the type of hearing loss. The other attributes concerned duration of hearing loss, fluctuation of hearing, caloric asymmetry, and occurrence of sudden falls.

The sudden deafness cases (n = 6) that the best decision tree, constructed from all cases, did not find had much missing information and the absence of vertigo, which interfered with the decision-making. These cases were grouped as belonging to either vestibular schwannoma or Meniere's disease. There was only 1 false-positive case, which actually was vestibular schwannoma.

Traumatic Vertigo. The mean estimated accuracy for traumatic vertigo was 99%. This high accuracy was reached with 53 questions. The most important question in the trees was the occurrence of head injury in relation to the time of onset of vertigo. This was the root attribute in each one of the decision trees. The only other attribute in the decision trees concerned the duration of vertigo attacks.

There were 2 cases of traumatic vertigo that were not classified correctly. The first false positive was typical benign positional vertigo, but the relation of time of onset to head trauma was unclear. The other patient had the full triad of vertigo, hearing loss, and tinnitus mimicking Meniere's disease, which could be the sequel of a traumatic event to the inner ear.

Vestibular Neuritis. The best estimated accuracy, 100%, was reached for vestibular neuritis. Both the false-positive rate and the true-positive rate for vestibular neuritis were good. There was only 1 case of vestibular neuritis that the decision tree did not find. This was due to missing information in relation to the frequency of vertigo attacks. There were also 2 false-positive cases classified as vestibular neuritis. Both patients had MRI-confirmed vestibular schwannoma, but otherwise symptoms typical of vestibular neuritis. The results of CT (or MRI) did not belong to the 53 attributes from which the trees concerning vestibular neuritis were constructed. The root attribute confirmed normal hearing in 8 of the 10 test cases. In the residual cases (2/10), the root attribute concerned the occurrence of vertigo attacks.

DISCUSSION

Decision trees were used to generate diagnostic rules from neurotologic data. The estimated accuracy of the rules was between 94% and 100% for all the diseases. The lowest score was 94% for Meniere's disease, where 110 attributes were used in the decision-making. The various clinical constellations make Meniere's disease difficult to diagnose. The structure of decision trees is clear and easily comprehended (Fig 1), and the information is useful also outside the expert system. The root attribute was essential in finding and classifying the disease cases. These key attributes were the presence of hearing loss, vertigo, and tinnitus; duration of vertigo; frequency of vertigo attacks; score for rotational vertigo; type of hearing loss; and occurrence of head injury in relation to the timing of the onset of vertigo.

The number of inner nodes in each decision tree represents the number of questions required for construction of the tree, and the number of leaves tells the number of valuable classification answer possibilities to these questions. In this study, the number of leaves in the decision trees was in direct relation to difficulty in assessing the diagnosis. The largest numbers of leaves were for Meniere's disease, the second largest for vestibular schwannoma, and the third largest for sudden deafness. For the other 3 diagnoses, the sizes of the decision trees were nearly the same.

We coded the discrete ordered attributes in 2 ways: discrete and continuous. In the discrete coding, each value of an attribute has a corresponding branch in a decision tree, whereas in continuous coding, a cutoff point is calculated for attribute values and an attribute has only 2 branches. Continuous coding resulted in the best decision tree in 4 diseases of the 6. Only in vestibular neuritis and in vestibular schwannoma was discrete coding better. Continuous coding resulted in a well-organized and easy-to-comprehend decision tree with fewer nodes and leaves.

The possibility of constructing a decision tree in an interactive manner would be advantageous. During the construction of a decision tree, a user could choose which attributes are to be used in the decision tree and which are to be left out. In this way it would be possible to generate different classification models and take advantage of a human expert's knowledge. It would aid in finding new diagnostically valuable factors, when the known factors could be reevaluated with stepwise analyses. It would also assist in ruling out some database-dependent variables, such as the duration of hearing loss and vertigo in our database.

The high accuracy in detecting each disease is worth noting. It is partly due to the organized approach of the program to problem-solving. It takes all available factors into consideration before making the decision. One deviant or otherwise inappropriate response to any parameter does not automatically exclude the disease. If all other information fits for the disease, it is most likely suggested. Human decision-making is typically a constant and deductive process, which often leads the questioning and, by doing so, affects the result of decision-making.[12] All relevant questions are not necessarily asked in this type of questioning.

Typical symptoms do not always reveal the expected diagnosis. The 2 patients with typical symptoms of benign positional vertigo who actually had confirmed vestibular schwannoma raise the question whether more MRI should be done in patients with clinically confirmed benign positional vertigo. Particularly if there is any neurologic abnormality, MRI is strongly advised. Dunniway and Welling[13] reported intracranial tumors with typical symptoms of benign positional vertigo.

The small size of the patient material and the small number of patients in rare disease groups make the use of machine learning techniques difficult, sometimes impossible. With decision trees, the number of solved cases need not be as high as in other machinery learning methods, like neural networks. This makes them advantageous. The cross-validation technique makes it easier to overcome the low number of cases and enables high reliability of the results. With highly imbalanced training sets, learning systems designed to optimize classification accuracy may produce classifiers that classify negative cases correctly but misclassify many positive cases.[14] In this material, the number of sudden deafness cases was low (n = 21), so the class distribution of positive cases and negative cases was highly unbalanced for sudden deafness. This may have resulted in a low true-positive rate for sudden deafness.

In medical domains, wherein new cases come daily, the ability to update a decision tree is favorable. The See5 classification system does not have this possibility. Thus, new cases have to be added to the training set and a new classifier has to be constructed from it. Algorithms for incremental decision tree induction have been developed, by Schlimmer and Fisher[15] and Utgoff,[16] for instance.

In this study, the key questions in classifying the 6 diseases concerned the occurrence of vertigo, hearing loss, and tinnitus; the duration of vertigo; the frequency of vertigo attacks; the score for rotational vertigo; the type of hearing loss; and the occurrence of head trauma in relation to the onset of symptoms. These questions agree well with our earlier findings on discrimination analysis of these 6 diseases.[8] The neurotologic tests are of minor value in the differential diagnosis.

CONCLUSION

Decision trees found, from a large database, attributes that best discriminated among 6 diseases involving vertigo. Decision trees were able to create accurate diagnostic rules for the 6 diseases. The diagnostic rules produced with decision trees are easily explored and productive in gaining new information for diagnostic work. Interactive decision tree production may produce even better rules.

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RHEUMATOID ARTHRITIS OF THE TEMPOROMANDIBULAR JOINT WITH HERNIATION INTO THE EXTERNAL AUDITORY CANAL

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Previous authors have shown that soft tissue can present in the external auditory canal via a patent foramen of Huschke. One case represented a patient with psoriatic arthritis and a polyp in the external auditory canal. Typically, neoplastic, inflammatory, or degenerative lesions of the temporomandibular joint do not present in the external auditory canal. We present a patient with rheumatoid arthritis of the temporomandibular joint and soft tissue herniation into the external auditory canal. The case, and a discussion of possible causes, are presented.

KEY WORDS — external auditory canal, rheumatoid arthritis, temporomandibular joint.

CASE REPORT

A 64-year-old man first presented in April 1997 with a complaint of hearing loss (right greater than left) that had gradually progressed over the past 2 to 3 months. He claimed that his hearing improved with Valsalva's maneuver. In 1992, he had had a similar episode that resolved with myringotomy and tube placement. His past medical history was positive for hypertension and significant rheumatoid arthritis, for which he took the following medications: sucralfate 2 mg twice per day, methotrexate 2.5 mg/wk, Fosamax 10 mg every morning, folate 1 mg/d, prednisone 6 mg/d, and tolmetin sodium 400 mg twice per day.

The physical examination was notable for advanced systemic signs of rheumatoid arthritis, namely, severe joint deformities to a degree necessitating C1-2 fusion, hip replacement, and numerous arthroscopic procedures. The otolaryngological examination was significant for an amber effusion in the left ear and a retracted tympanic membrane in the right.

When the patient returned for follow-up in June 1997, he still complained of minor fullness of the right ear. Examination revealed no effusions in either ear, and he was instructed to continue to use Valsalva's maneuver and return as needed.

In March 1998, the patient returned with the complaint of fullness of the right ear once again, but now with otorrhea. On examination, he had normal tympanic membranes, but clear fluid was emanating from an area of granulation tissue on the anterior external auditory canal wall, suggesting a salivary fistula. Computed tomography (CT) of the temporal bones showed a bony defect in the anterior wall of the external auditory canal (see Figure, A) and some irregularity of the posterior aspect of the mandibular condyle (see Figure, B) on the right. (The left side is presented for comparison in the Figure, C.) Magnetic resonance imaging was done due to concern regarding a possible malignancy of the external auditory canal or parotid gland, and it was negative.

On April 13, 1998, the patient underwent excision of the left external auditory canal lesion and reconstruction of the bony defect. During the procedure, frozen sections of the lesion yielded a diagnosis of granulation tissue. The dehiscent area of the anterior external auditory canal was explored and followed to the temporomandibular joint without any evidence of a mass. The final pathologic diagnosis consisted of fibrotic granulation tissue with areas of necrosis. On the basis of the pathology and CT findings, the diagnosis of rheumatoid arthritis of the temporomandibular joint was made. Postoperatively, there was no residual granulation tissue, but the fascia used during the reconstruction was herniating into the external auditory canal and partially occluding it. Steroid injection of the temporomandibular joint has resulted in complete resolution of the soft tissue herniation.

DISCUSSION

The most common systemic disease affecting the temporomandibular joint is rheumatoid arthritis. Rheumatoid arthritis is an autoimmune inflammatory condition in which inflamed and hypertrophic synovial membrane grows on the articulating surfaces of joints. The presence of such vascularized and innervated tissue on pressure-bearing surfaces causes pain and inflammation with normal functioning of the joint. Rheumatoid arthritis is extremely variable in incidence, severity, and clinical course.

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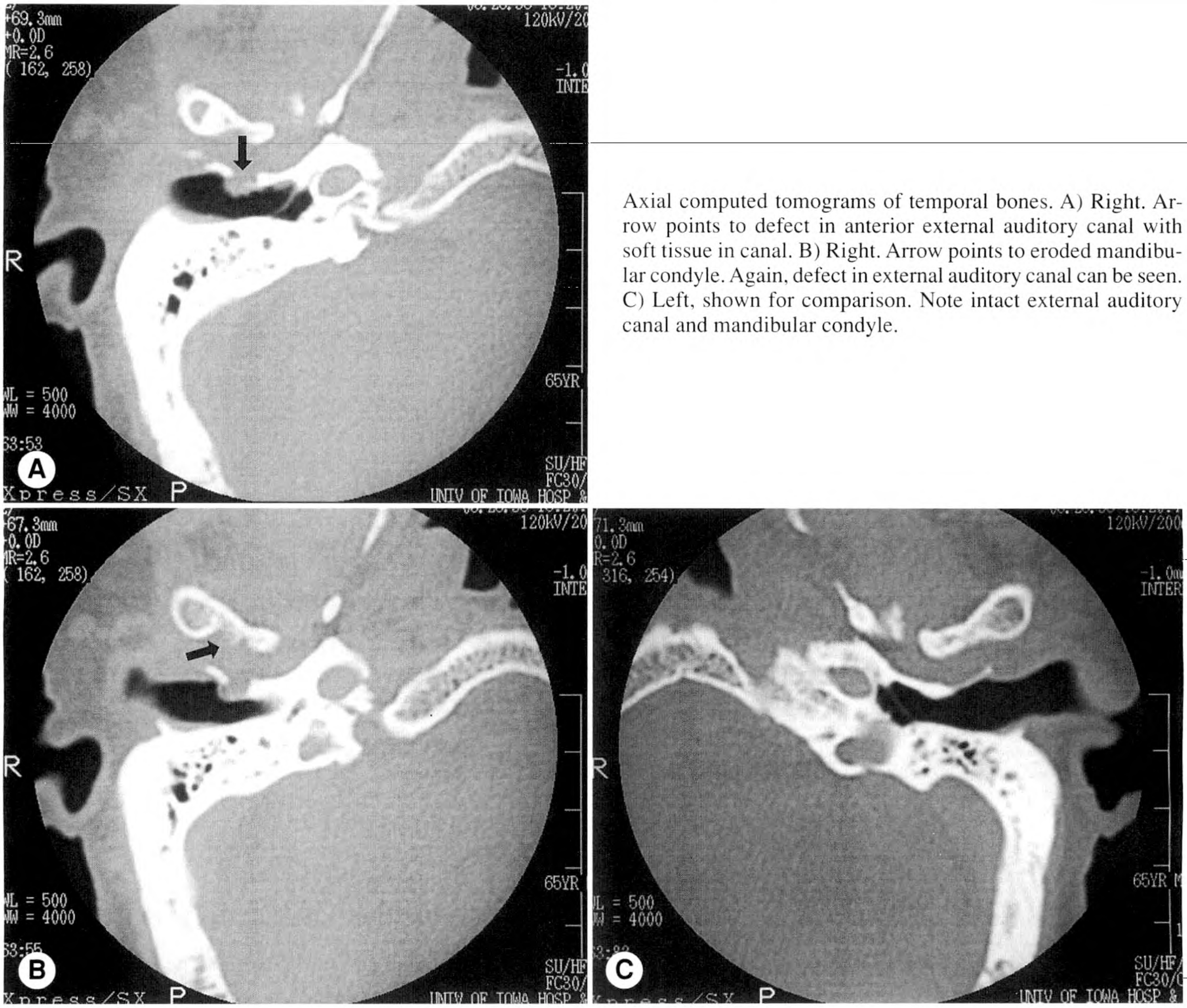

Axial computed tomograms of temporal bones. A) Right. Arrow points to defect in anterior external auditory canal with soft tissue in canal. B) Right. Arrow points to eroded mandibular condyle. Again, defect in external auditory canal can be seen. C) Left, shown for comparison. Note intact external auditory canal and mandibular condyle.

The incidence of temporomandibular joint involvement in rheumatoid arthritis is substantial. Haandes et al[1] reported that greater than half of rheumatoid arthritis cases involve the temporomandibular joint.

The first case in the literature of temporomandibular joint tissue herniation into the bony external auditory canal was secondary to trauma.[2] The first case of spontaneous herniation was reported by Hawke et al.[2] One year later, they reported the first case of a spontaneous fistula between the joint and the external auditory canal.[3]

Congenital dehiscence of the anterior external auditory canal wall has been attributed to the persistence of the foramen of Huschke. As the edges of the tympanic bone grow toward each other, during development, they fuse lateral-to-medial, creating a foramen in the medial portion of the canal wall anteroinferiorly. The foramen of Huschke usually closes by the fourth to fifth year of life, but may persist into adult life. Wang et al[4] examined 377 adult skulls and found the rate of persistence of the foramen of Huschke to be 7.2%, with a bilateral incidence in 14 skulls. The reported incidence varies between 5% and 46%.[4] This defect would allow access of surrounding structures (temporomandibular joint, parotid, etc) to the external auditory canal.

Neoplastic, inflammatory, and degenerative lesions of the temporomandibular joint do not typically present in the external auditory canal, especially those of arthritic diseases. A patent foramen of Huschke could allow such a presentation. In 1991, Cecire et al[5] presented a case of a polyp of the external auditory canal arising from the temporomandibular joint in a patient with psoriatic arthritis and a patent foramen of Huschke. This is quite similar to our case.

A thorough search of the literature did not reveal any reported cases of a mass of the external auditory canal attributable to herniation of the temporomandibular joint affected with rheumatoid arthritis. This would be the first reported case of the kind. In this case, there are 2 possible explanations for the external auditory canal findings. First, the patient could have had a patent foramen of Huschke on the left, which was small enough that there was no herniation of temporomandibular joint or any other soft tissue contents into the external auditory canal. Also, intervening soft tissue between the external auditory canal and the temporomandibular joint could prevent herniation into the canal. Over time, pannus from the disease process could have softened the intervening tissue or enlarged this opening to a size sufficient to allow the soft tissue herniation seen on clinical examination. This would explain why no mass was seen in the external auditory canal when the patient first presented in April 1997. Second, this could represent primary erosion of the tympanic ring by the rheumatoid arthritic disease process with herniation of soft tissue into the canal as a secondary event, which has not been previously described.

There are data to support the latter supposition. In the article by Wang et al,[4] 31 foramina of Huschke were measured, with the average coronal diameter being 2.7 mm (1.0 to 8.0) and the sagittal diameter 3.0 mm (1.0 to 6.0). This is smaller than the dimensions encountered during surgical exploration, which were approximately 10 × 15 mm (5 × 5 mm on CT). Also, of the 41 foramina observed, the medial margin of the foramen was very close (less than 0.5 mm) to the tympanic sulcus in 39 specimens and was separated by a ridge of bone, never actually crossing this structure.[4] In our case, the medial margin was at least 2 mm from the sulcus.

In the end, neither scenario can be definitively proven. There do seem to be striking similarities between the case presented by Cecire et al[5] and ours. In both, there is an underlying rheumatologic arthritic process present in the patient. Because the patient in the Cecire et al article presented with a polyp in the external auditory canal, similar to other cases of a patent foramen of Huschke, this could represent a spontaneous herniation or a herniation secondary to arthritic erosion of bone. Our patient developed a soft tissue mass several months after initial presentation, so either of the 2 theories could be responsible for the mass in our patient.

The above case only highlights what has been presented before, in that the differential diagnosis of lesions of the external auditory canal should include not only conditions that may arise from the canal itself, but also lesions arising from surrounding structures.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF COLLAGEN TYPES I, III, AND IV, LAMININ, FIBRONECTIN, AND KERATIN IN THE ENDOLYMPHATIC SAC

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We have employed immunohistochemistry to obtain baseline information on the molecular constituents of the extracellular matrix (ECM) of the endolymphatic duct (ED) and endolymphatic sac (ES) of the chinchilla. The results demonstrated that collagen types I and III were distributed in the subepithelial layer in the ED and ES, type IV collagen and laminin in the basement membranes, and fibronectin in the subepithelial layer and partly in the conglomerated cells in the ES. Collagen type III was diffusely distributed in the whole subepithelial layer of the ES, whereas collagen type I was concentrated densely in the deep layer of the interstitium, although gradually, the cuboidal epithelium in the ES was transformed into a flatter type in the ED. The epithelial cells of the ED and ES were clearly positive for keratin. This study deals, in particular, with the normal distribution of ECM components of the ED and ES of the chinchilla.

KEY WORDS — endolymphatic sac, extracellular matrix, immunohistochemistry, keratin.

INTRODUCTION

The endolymphatic duct (ED) and endolymphatic sac (ES) are the terminal portions of the endolymphatic system. The ES is believed to have a resorptive and phagocytic function. These structures may participate in the regulation of the amount of fluid and of the ionic composition in the endolymphatic compartment by draining endolymph through the epithelial lining to the surrounding vessels.[1] The secretion of high–molecular weight substances has also been suggested to be important for the regulation of endolymph volume and pressure.[2,3]

It is widely accepted that the extracellular matrix (ECM), which comprises a well-organized complex of collagens, elastin, proteoglycans, and glycoproteins such as fibronectin and laminin, can directly influence cell behavior and may modulate cell function in several tissues.[4] Few studies have focused on the ECM of the ES. The precise distribution and function of ECM components in the ES has not yet been determined.

Epithelial cell keratins are a complex group of intermediate filament proteins.[5] The overall pattern of keratin expression by diverse epithelia is thought to reflect specific location and differentiated cellular function.[6] Studies have demonstrated that the state of differentiation of epithelial cells in vitro is influenced by the ECM on which they are cultured.[7]

As the first step, the goal of this study was to anatomically localize the ECM components, which may be involved in not only proliferation and differentiation of the epithelial cells but also in fluid-ion permeability regulation, and to localize keratin in the normal ES by means of immunohistochemistry.

MATERIALS AND METHODS

Animals. Twenty normal chinchillas, approximately 1 year old, were used in this study. The animals were anesthetized with a combination of ketamine hydrochloride (90 mg/kg, intramuscularly) and sodium pentobarbital (20 mg/kg, intramuscularly). The ED and ES were taken out by the posterior fossa approach. In addition, chinchilla kidney and external ear skin were histologically processed with the ED and ES to serve as positive controls. Tissue was snap-frozen in isopentane precooled in liquid nitrogen, and sectioned at 4 μm in a Lipshaw cryostat (Lipshaw Manufacturing, Co, Detroit, Mich).

From the University of Minnesota Otitis Media Center (Harada, Juhn, Kim) and the Departments of Otolaryngology (Harada, Juhn) and Pediatrics (Kim), University of Minnesota, Minneapolis, Minnesota, and the Department of Otorhinolaryngology, Mie University School of Medicine, Tsu, Japan (Harada, Sakakura). This study was supported by program project 8P50-DC-00133 from the National Institutes of Health/National Institute on Deafness and Other Communication Disorders. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

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TABLE 1. PRIMARY AND SECONDARY ANTIBODIES FOR IMMUNOHISTOCHEMISTRY

Primary Antibody	*Source*	*Dilution*	*Secondary Antibody*	*Source*	*Dilution*
Collagen type I	SB	1:32	FITC-RAGG	Cappel	1:10
Collagen type III	SB	1:64	FITC-RAGG	Cappel	1:10
Collagen type IV	SB	1:64	FITC-RAGG	Cappel	1:10
Laminin 86-9	MN	1:128	FITC-GARG	Cappel	1:32
Fibronectin	SB	1:32	FITC-GARG	Cappel	1:32
Keratin	Miles	1:32	FITC-GAGPG	Cappel	1:16

SB — Southern Biotechnology Associates, Inc, Birmingham, Ala; MN — provided by the Dept of Pediatrics, University of Minnesota; Miles — Miles Laboratories, Inc, Naperville, Ill; FITC — fluorescein isothiocyanate; RAGG — rabbit anti–guinea pig IgG antiserum; GARG — goat anti-rabbit IgG antiserum; GAGPG — goat anti–guinea pig IgG antiserum; Cappel — Cappel, Organon on Teknika Corporation, West Chester, Pa.

Reprinted from Harada T, Juhn SK, Kim Y, Sakakura Y. Immunohistochemical distribution of extracellular matrix components and keratin in experimentally induced otitis media. Ann Otol Rhinol Laryngol 1999;108:769-76.

Immunohistochemistry. An indirect immunohistochemical method was used as previously described.[8] In brief, the slides were rinsed in phosphate-buffered saline (PBS; 3 changes, 3 minutes per change) and overlaid with approximately 20 µL of an appropriate dilution of primary antiserum. The sources of primary and secondary antibodies and their appropriate dilutions are shown in Table 1. Each antibody solution was titered on tissue, and the concentration that gave maximal specific fluorescence and minimal background fluorescence was used for these studies. All primary antibodies were polyclonal. Tissue sections were incubated in the primary antibody for 30 minutes in a dark, moist chamber at room temperature. Preincubation of the sections in 1% bovine serum albumin in PBS before the primary and/or secondary antibody had little effect on the staining specificity or background; therefore, it was usually omitted from the solutions. Sections were then rinsed in PBS (3 changes, 3 minutes per change) and incubated in a fluorescein isothiocyanate (FITC)–labeled secondary antibody for 45 minutes in a dark, moist chamber at room temperature. Sections were washed in PBS (3 changes, 3 minutes per change) and mounted in glycerol containing *p*-phenylenediamine to slow bleaching of the FITC staining. Secondary antibodies were absorbed with chinchilla serum before use. Sections were examined with a Zeiss phase contrast microscope with epifluorescence illumination. The distribution and intensity of immunofluorescence was scored on a semiquantitative scale of trace, moderate, strong, and very strong fluorescence. Photographs were taken with Kodak T-max film, rated at ISO 1600.

Two immunohistochemical controls were performed. First, chinchilla kidney and external ear skin were used to serve as a positive control and to determine whether the antibodies would cross-react with chinchilla tissues even though the antibodies were raised against human antigens. Although these primary antibodies react with human ECM components, they also react specifically with ECM components from most other species. These antibodies also react specifically with ECM components from chinchilla. These tissues were processed identically as the ED and ES. Second, as a negative control, tissue sections were incubated only in the secondary antibody.

TABLE 2. DISTRIBUTION OF EXTRACELLULAR MATRIX COMPONENTS AND KERATIN IN ENDOLYMPHATIC DUCT AND SAC OF CHINCHILLA

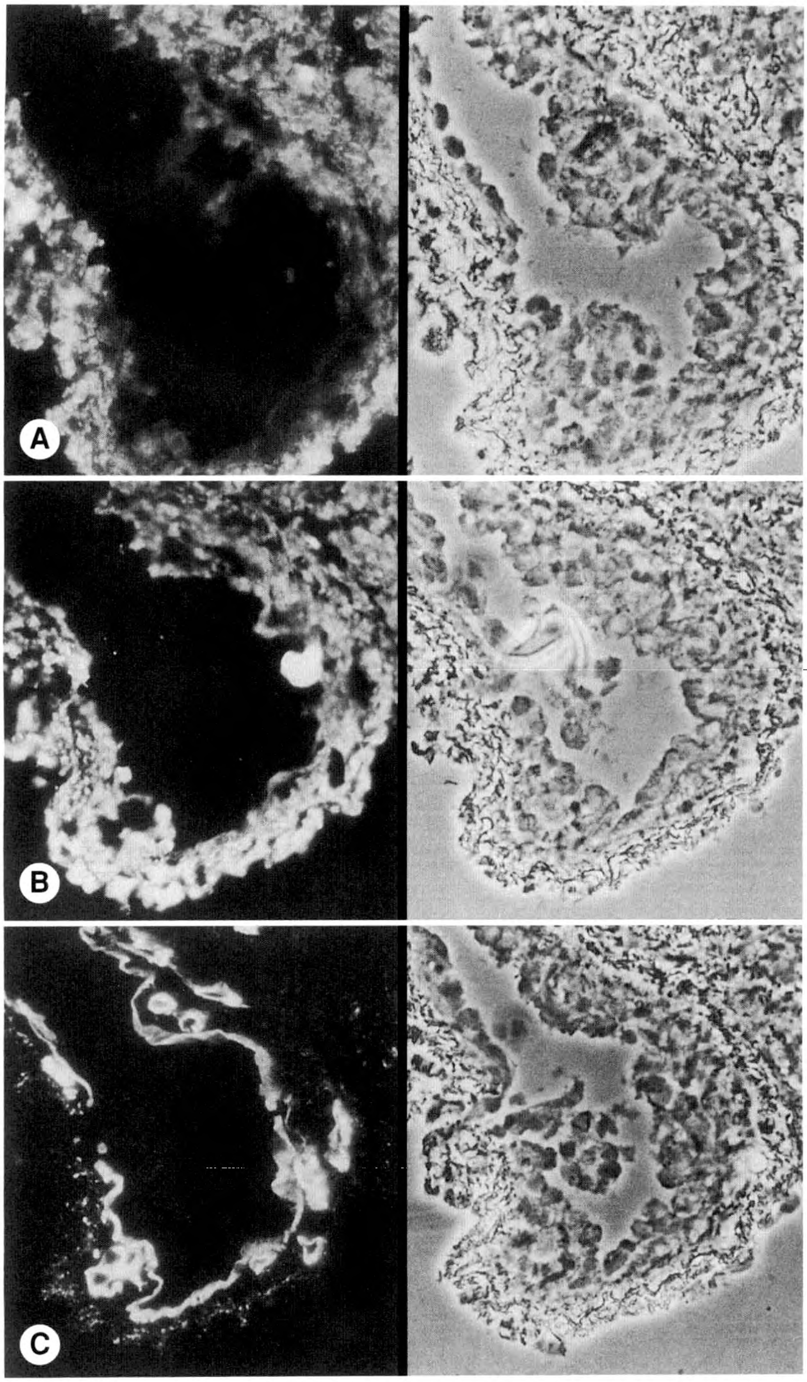
	Collagen Type I	*Collagen Type III*	*Collagen Type IV*	*Laminin*	*Fibronectin*	*Keratin*
Endolymphatic sac						
Epithelial cells	–	–	–	–	–	± to +*
Basement membrane	–	–	++†	++†	–	–
Subepithelial layer	++	++§	–	–	++	–
Endolymphatic duct						
Epithelial cells	–	–	–	–	–	± to +*
Basement membrane	–	–	++†	++†	–	–
Subepithelial layer	++	++§	–	–	++	–

Immunofluorescence was scored as follows: – — none; ± — weak; + — moderate; ++ — marked. When scores of 2 observers differed, result is given as range.

*Epithelial cells in endolymphatic sac are polymorphous; cuboidal to wedge-shaped. Those in endolymphatic duct are flat, often triangular.

†Collagen type IV and laminin were distributed in not only basement membrane of epithelium but also that of capillaries in subepithelial layer.

§Collagen type III was diffusely distributed in whole subepithelial layer of endolymphatic sac, while collagen type I was located more in deep layer of interstitium.



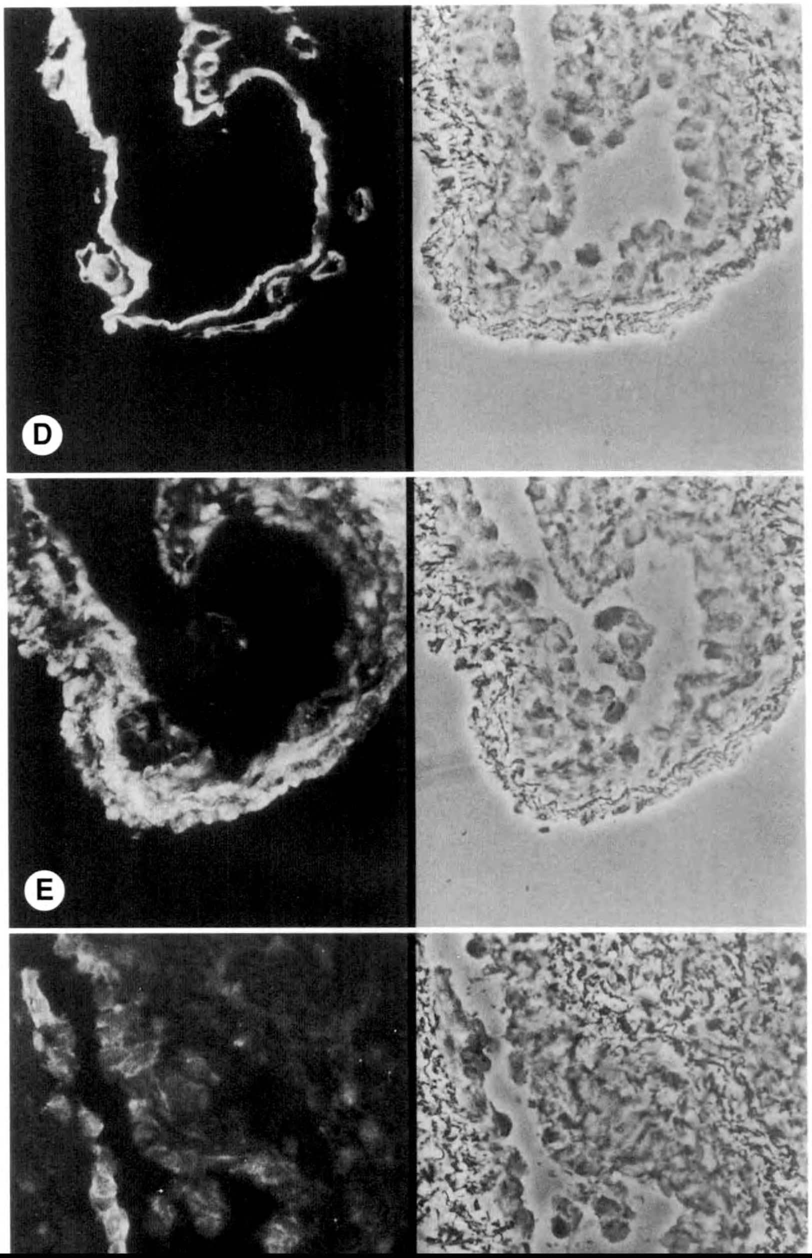
Normal chinchilla endolymphatic sacs subjected to immunofluorescence (left) and phase-contrast microscopic picture of same region (right). A) Collagen type I. B) Collagen type III. C) Collagen type IV. (Continued on next page.)

Appropriate controls were always used as previously described.[8] The staining intensity was graded as follows: no staining (−), weak staining (±), moderate staining (+), and marked staining (++).

RESULTS

The reactivity patterns of the different ECM antibodies and the keratin antibodies on normal chinchilla ED and ES sections are summarized in Table 2 and depicted in the Figure.

Collagen types I and III were present throughout the subepithelial layer (see Figure, A,B). These 2 collagens were found to be dense in the extraosseous ES, at the junction of the external aperture of the vestibular aqueduct and the beginning of the extraosseous ES. They were found to be loosely distributed in the subepithelial layer close to the basement membrane. Collagen type III was diffusely distributed in the whole subepithelial layer of the ES (see Figure, B), while collagen type I was located, denser, in the deep layer of the interstitium (see Figure, A). The subepithelial layer of the intraosseous ES and ED had a thin layer of collagen types I and III.



(Continued.) D) Laminin. E) Fibronectin. F) Keratin.

[redacted]

DISCUSSION

The localization of 5 ECM components in the chinchilla ED and ES was determined by use of the immunofluorescence technique. For this study, we selected 2 known basement membrane constituents (collagen type IV and laminin) and 3 more broadly distributed constituents (collagen type I, collagen type III, and fibronectin). Our data showed that the localization of these ECM components was distinctive in all tissues examined. Collagen type IV and laminin stained basement membranes very strongly. Fibronectin, as well as collagen types I and III, displayed a broader distribution in the connective tissue of the ED and ES (Table 2).

Basement membranes are thin ECM components with a ubiquitous occurrence in the body, and their close apposition to cells is their most remarkable feature. These cell contacts are mediated by cellular receptors and specific ECM components. The extracellular matrix has profound effects on polarization, differentiation, and proliferation of cells and on the control of their migratory behavior. In addition, basement membranes are barriers regulating filtration of macromolecules and penetration by cells. The components of basement membranes include collagen type IV, the cell-binding protein laminin, several proteoglycans, and other proteins.[4] The distribution of collagen type IV and laminin in the chinchilla ES was similar to that reported in the ES of guinea pigs.[9] Collagen type IV and laminin were localized around the vascular and epithelial basement membranes.

The distribution of collagen types I and III was similar to that reported in other organs.[10] These collagens were restricted to the interstitium (see Figure, A,B). Collagen type III was diffusely distributed throughout the whole subepithelial layer (see Figure, B); collagen type I was concentrated more in the deep layer of the interstitium (see Figure, A). Fibroblasts synthesize and secrete predominantly collagen types I and III; the latter accounts for a small proportion, varying from 5% to 30%, of the total secreted procollagen.[11] The role of collagen deposition by the fibroblasts during wound repair is well established. Granulation tissue, as well as fetal skin, contains almost exclusively collagen type III, whereas adult skin contains mainly collagen type I. Therefore, it is likely that enhanced collagen type III synthesis and deposition will be found in a variety of inflammatory states and in response to diverse injurious agents. Substantial changes in the distribution of these collagens and of fibronectin are reported to be associated with the cellular differentiation and the morphogenetic events in the embryonic tooth.[10] Because of the prevalence of collagen type III in pliable tissues such as lung, blood vessel, and uterus, it has been suggested that it may have an important role in maintaining tissue pliability.

The relationship between histopathologic changes in the ED and ES and the pathogenesis of Meniere's disease is still contradictory and inconclusive. Schindler et al[12] found increased deposition of collagen both inside the lumen and in the perisaccular tissue. Barbara et al[13] studied the effects of an impaired body fluid metabolism on the inner ear fluid environment in the Mongolian gerbil and found an increased looseness of the perisaccular tissue after ethacrynic acid treatment. As collagen plays a large number of structural, mechanical, and functional roles in animals and humans,[4] it is important to know the distribution of the ECM components such as collagen types I, III, and IV. Modulated synthesis of the various collagens may, therefore, be crucial for the normal progression of dynamic processes such as development, tissue repair, and fluid metabolism in the inner ear fluid environment. Although we have not studied the distribution of collagen type II in this study, Yoo et al[14] reported type II autoimmunity as a cause of endolymphatic hydrops.

Two types of fibronectin are recognized.[15] Cell surface or cellular fibronectin is synthesized by a variety of cells, including fibroblasts, myoblasts, and certain epithelial cells. Plasma fibronectin is a dimer of 2 disulfide-bonded polypeptides, whereas cellular fibronectin occurs as dimers and multimers. Most antisera cross-react with the 2 forms of fibronectin. The antiserum that we used in this study also cross-reacted with both types of fibronectin. Monoclonal antibodies that distinguish between the types of fibronectin, however, have recently been available through several commercial sources. Structural differences between plasma fibronectin and cellular fibronectin have been well documented by molecular cloning of fibronectin complementary DNA.[16] The primary function of cellular fibronectin is to mediate the adhesion of cells to the ECM and to other cells.[4] The most obvious role for fibronectin in the ED and ES may be to provide structural support for the collagen fibers while allowing for the permeability of certain ions to or from the lumen of the ED and ES. Small ions such as potassium rapidly pass through the epithelium in the ED and ES.[17] Although it is not clear whether fibronectin is a mitogen for epithelial cells in the ED and ES, it is known to affect proliferation of epithelial cells by mediating their anchorage.[18]

Mesenchymal cells are elongated or stellar in shape in vitro, and they give rise to fibroblasts, chondrocytes, and similar structures. A mesenchymal cell migrating through the ECM is bipolar in shape, makes minimal intercellular contacts, and interacts with the ECM on all sides.[19] Mesenchymal cells typically make collagen type I and other structural glycoproteins that make up the interstitial matrix, whereas epithelia usually synthesize the basement membrane (basal lamina). Epithelial cells also produce intermediate filaments of the cytokeratin type.

The epithelia of the ED and ES are thought to play an important role in regulating inner ear fluid.[1] The characteristic components of intermediate filaments in epithelial cells and cells of epithelial origin are the keratins. The keratins comprise a highly complex family of polypeptides.[20] Although we showed the distribution of keratin using polyclonal antiserum, the keratins have a number of polypeptide subunits. Several lines of evidence indicate that the program for keratin synthesis can be altered when the environment of the cell is changed. In fact, Anniko et al[21] reported the distribution of cytokeratins 8 and 18 in cells lining the ED and ES of mice. They found immunoreactivity for cytokeratin 18 apically in cells lining the ED, whereas cytokeratin 8 was present throughout the cell cytoplasm.[21] Bauwens et al[22] reported that the epithelium of the ED and ES demonstrated coexpression of cytokeratins 7, 8, 14, 17, 18, and 19 and vimentin. Dahlmann and von During[23] have recently reported that 2 different types of epithelial cells could be identified in the ED and ES by electron microscopy. These 2 cell types are the ribosome-rich cell and the mitochondrion-rich cell. The epithelial cells in the ES are mainly cuboidal, whereas those in the ED are flat or squamous.[23] These results were in good agreement with those reported in humans.[24] All epithelial cells react with antikeratin antibody. More keratin expression patterns have to be examined in the ED and ES under physiological and pathological conditions, because keratin filaments are thought to play roles in controlling cell shape, cell growth, cell association, and cell mitosis.[25]

This study has provided baseline information on certain ECM components and keratin and their distribution within the ED and ES tissues. It is highly likely that within the ED and ES many different types of ECM components exist, with each serving a different function. The importance of these ECM components for normal ES function is just beginning to be understood.

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MIDLINE LATERALIZATION THYROPLASTY FOR ADDUCTOR SPASMODIC DYSPHONIA

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Midline lateralization thyroplasty was successfully performed on a patient with adductor spasmodic dysphonia. The thyroid cartilage was incised at the midline, and a 3 × 2-mm perforation was made at the anterior commissure to widen it. The perforation was closed with a free composite graft taken from the upper edge of the thyroid ala, and the incised thyroid cartilage edges were kept separated 4 mm apart with silicone wedges. A part of the sternohyoid muscle was rotated to seal any leak from the perforation. The postoperative course was uneventful. The voice has been restored to normal, and there is no sign of recurrence of the symptom so far, as of 1 year 5 months postoperative. Although a longer follow-up is needed, this case indicates that midline type II thyroplasty could be a useful treatment for adductor spasmodic dysphonia.

KEY WORDS — adductor spasmodic dysphonia, midline lateralization thyroplasty.

INTRODUCTION

Spasmodic dysphonia remains one of the most inveterate dysphonias, despite various attempts to treat the disease. A recurrent laryngeal nerve section for adductor spasmodic dysphonia,[1-4] first introduced by Dedo[1] in 1976, gained significant success, but its use gradually declined due to a high late-recurrence rate as reported by Aronson and DeSanto[4] and the inherent disability that was incurred. Fritzell et al[5] ascribed the recurrence to nerve regeneration.

Selective section of the adductor branch of the recurrent nerve was performed by Carpenter et al[6] and Iwamura (unpublished observations). Attributing the recurrence mainly to nerve regrowth, Netterville et al[7] developed a recurrent nerve avulsion for spasmodic dysphonia, and their group, Weed et al,[8] reported their long-term recurrence rate to be as low as 11%. Botulinum toxin injection,[9-19] first applied to laryngeal dystonia by Blitzer et al[10] and Miller et al,[9] has become the treatment of choice, due to its high success rate in restoring or improving the voice, less surgical intervention, and only temporary deprivation of vocal fold mobility. The disadvantages of the latter method are said to be that 1) repeated intracordal injections are often required, because the average duration of benefit is 4 months,[12] and 2) the injection site and dosage are difficult to determine, and this uncertainty occasionally results in temporary dysphonia due to imperfect closure of the glottis immediately after the injection.

The cause and pathological entity of spasmodic dysphonia remain mostly unknown, though recent reports[20-32] increasingly indicate some organic involvement of the central nervous system. The above treatments so far employed are, in any case, symptomatic and not curative. In view of the not always satisfactory results of the above treatments, Morrison and Rammage[33] emphasized the need for differential diagnosis between spasmodic dysphonia and laryngeal muscular tension dysphonia.

Our recent experience with midline type II thyroplasty (lateralization thyroplasty) performed on a female patient with spasmodic dysphonia showed promising results, and the case is detailed here.

PATIENT

A 40-year-old woman visited our clinic (Isshiki Voice Laboratory) complaining of phonation difficulty and a strained, occasionally almost aphonic, voice that had persisted for 1½ years. She could not sing, and using a telephone was very difficult. Her voice was better during sustained vowel phonation than during conversation, and yet still very rough, hoarse, and unstable. The onset of symptoms was about 9 months after the Great Hanshin Earthquake, which severely hit her residential area in Higashin-

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An outline of this paper was presented at the Thyroplasty Workshops at the Mayo Clinic, Rochester, Minn, October 24-26, 1997, at Free University Hospital, Amsterdam, the Netherlands, February 12-13, 1998, and at the meeting of the American Laryngological, Rhinological and Otological Society, Palm Beach, Florida, May 11-13, 1998.

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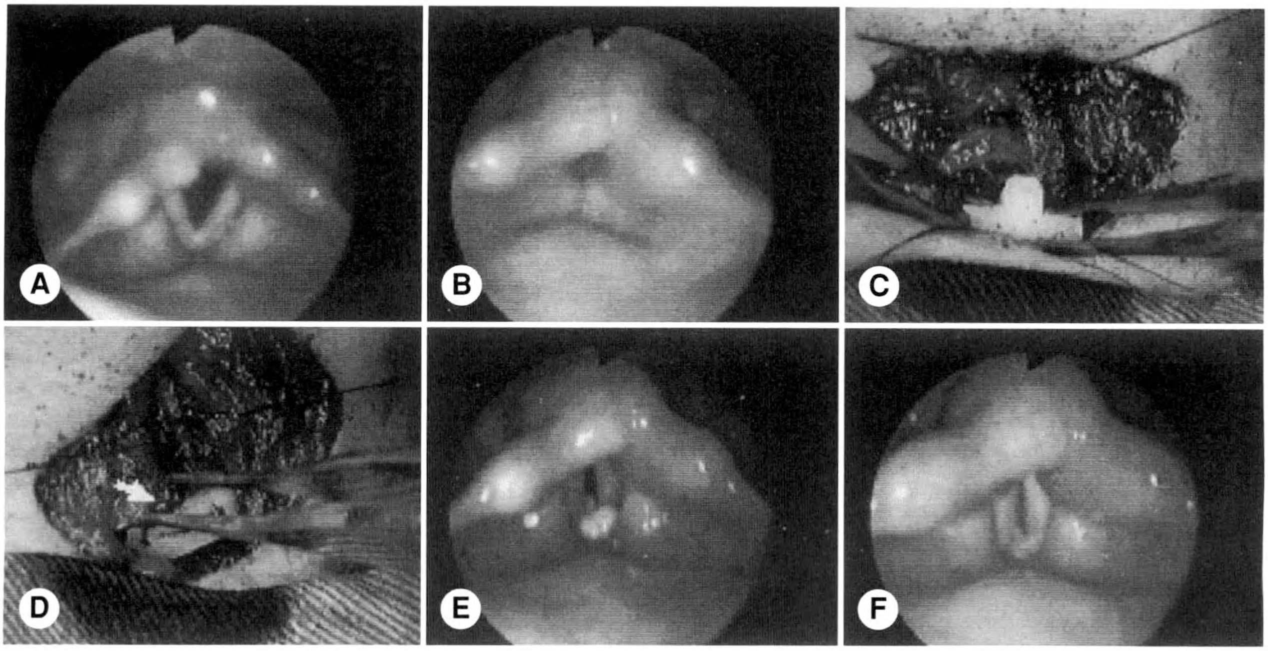

Fig 1. Laryngeal and intraoperative findings. A) Preoperative laryngeal findings during respiration. B) Preoperative findings during phonation. Note false vocal folds adducting, touching each other, and covering true vocal folds. C) Intraoperative view showing shim. Central projecting portion was later thinned to 3 mm to match thickness of cartilage. Shim shown is before further thinning. D) Intraoperative view showing sternohyoid muscle flap (arrow). E) One month postoperative laryngeal findings during phonation. Note white mass, protruding from anterior commissure, which did not hinder voice production. F) Three-month postoperative laryngeal findings during phonation. Mass has disappeared.

ada, Kobe, but she absolutely denied that the earthquake precipitated the dysphonia and denied any other psychological basis as well. The treatments she had received at several institutions so far, including a short-term trial of voice therapy employing the muscular relaxation procedure of Roy et al[34] and the administration of minor tranquilizers, all failed to improve her voice. She complained of neither gastroesophageal (laryngopharyngeal) reflux nor a lump in the throat in connection with the vocal symptoms, but in the course of the follow-up examination, her gag reflex was found to be very sensitive. Findings of systemic and blood examinations were all within the normal range, except for a slightly high cholesterol level. Neural diseases such as Parkinson's disease, essential tremor, and other central nervous system diseases were all ruled out by neurologists.

Laryngofiberscopy revealed an excessively tight closure of the anterior glottis, with the false vocal folds touching each other at the midline, completely covering the vocal folds, and a posterior glottal chink remaining on phonation (Fig 1B). The left vocal fold appeared slightly more edematous than the right, though this finding disappeared 1 year after the operation. Neither inflammatory signs nor tumors were found (Fig 1A). Further follow-up for 3 months with conservative treatments such as voice therapy and psychotherapy failed to detect any change in the voice. She consented to our therapeutic recommendation, surgical widening of the anterior glottis.

SURGICAL TECHNIQUE

The surgery was performed under local anesthesia, and the surgical process together with the voice was recorded on videotape. A 4-cm horizontal skin incision was made at approximately the level of the vocal folds. The anterior third of the thyroid cartilage was fully exposed and cut at the midline with a BP 11 scalpel, and with a fine cutting bur for the inferior calcified portion (Fig 2A). Utmost caution was taken not to cut through the underlying soft tissue, to prevent inadvertent cutting of the anterior commissure or anterior portion of the vocal fold. The incised edges were pulled apart laterally to test the effect of lateralization on the voice, and further lateralization of the soft tissue by opening at the anterior commissure was thought necessary to ensure easy voice production and to prevent recurrence. As preoperatively planned, an injection needle was inserted at the point thought to be the anterior commissure projection for topographical confirmation, and a slight further lateral pull of the incised cartilage edges, without incision of the soft tissue, resulted in a perforation 3 × 2 mm in size exactly at the anterior commissure, because only a very thin piece of soft tissue had remained there.

In order to close the perforation, a composite graft was harvested from the upper margin of the left thy-

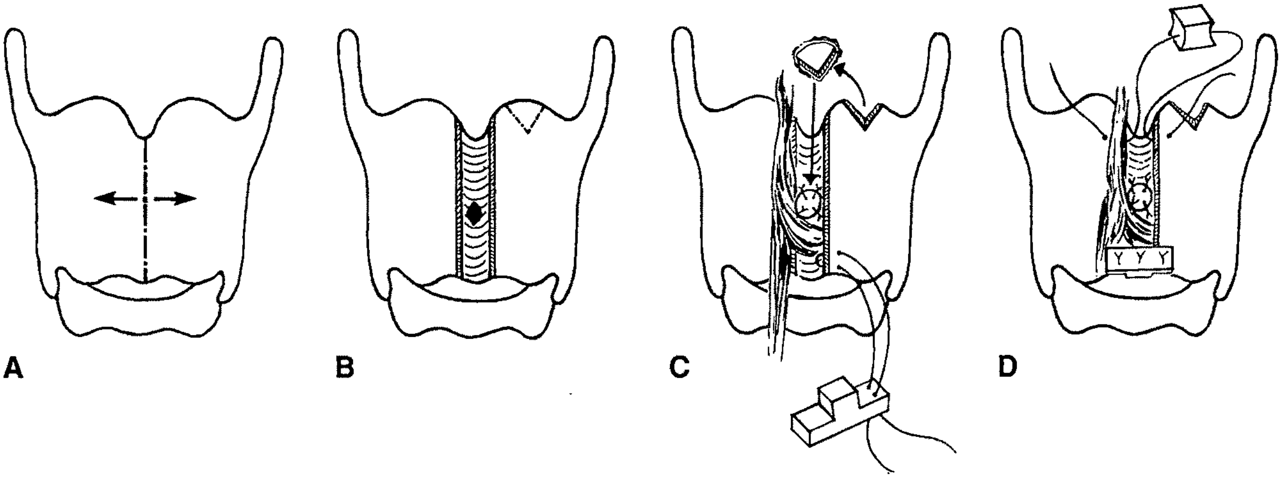

Fig 2. Surgical procedures. A) Incision of thyroid cartilage, with care not to cut through underlying soft tissue. B) Incised cartilage edges are pulled laterally and tiny hole is made at anterior commissure. Composite graft is to be harvested from upper edge of thyroid ala. C) Perforation is closed with graft, and portion of sternohyoid muscle is rotated and used as flap to ensure separation of perforation from shim below. D) Upper and lower shims separate incised cartilage edges 4 mm apart.

roid ala. It incorporated perichondrium on both sides for better viability, and some soft tissue inside as well, for a tighter seal of the perforation from the airway.

Before closure of the perforation, the required width of midline cartilage separation was determined, 4 mm in this case, and a silicone shim was fabricated to that size (Figs 1C and 2C) and threaded with 4-0 nylon sutures for later fixation to the thyroid cartilages.

The composite graft with the soft connective tissue facing inside was used to seal the perforation with four 6-0 nylon sutures (Fig 2C). In an attempt to ensure the sealing of the perforation so that the shim would not be contaminated by mucus leaking from the perforation, and to secure the contact of the free composite graft with the live covering tissue, a median portion of the sternohyoid muscle was cut caudally and its stump was rotated medially and sutured between the graft and the silicone shim (Figs 1D and 2C).

The shim was fixed to the incised edges of the thyroid cartilage with two 4-0 nylon mattress sutures, and another mattress suture was applied at the midportion of the shim to eliminate a possible dead space between the shim and the underlying soft tissue (Fig 2D). To maintain the cartilage separation in the upper part, a smaller and simpler shim was used, as shown in Fig 2D, because the incised cartilage edges were already fairly well stabilized by the lower shim. The operation was concluded when unstrained voicing with a normal sound was confirmed.

RESULTS

The postoperative course was uneventful. Four weeks after the operation (Fig 1E), the voice sounded nearly normal and completely free from strain, despite a tiny (about 1 mm) white pedunculated mass that was noted at the anterior commissure, flapping in synchrony with the flow of air in and out. This tiny necrotic mass, apparently derived from the connective tissue attached to the perichondro-chondrograft, gradually decreased in size, and had almost totally disappeared by 3 months (Fig 1F). Judging from the size, softness, and gradual shrinking process (not dropping), we think it was undoubtedly the connective tissue, and not the composite graft itself. That no red granulation tissue was noted at the anterior commissure indicated a good take of the graft. Since the mass was soft and above the vocal folds, it did not seem to have disturbed the vibration of the vocal folds. During phonation, the glottal closure was not tight, the vocal folds were visible as in normal phonation, and the anterior ends of the false vocal folds separated widely (Fig 1E,F), in contrast to the preoperative findings (Fig 1B).

As of postoperative 1 year 5 months, there is no sign of recurrence, and no strain perceptible in either the voice or the facial expression. Sound spectrographic analysis confirms that the voice is normal both in quality and pitch, with around 300 Hz in pitch for sustained phonation of /a/, in contrast to being extremely strained and hoarse (no pitch detectable) before the operation (Fig 3). As an index to express the severity of spasmodic dysphonia, the *morae* (units of Japanese speech in terms of both time and phonetic events, corresponding to syllables in English) that were judged to be distorted were counted before and after the operation, as proposed by Kumada and Kobayashi.[35] The number of distorted *morae* decreased dramatically, from 18 out of a total 21 *morae* preoperative, to 0 postoperative. The patient has

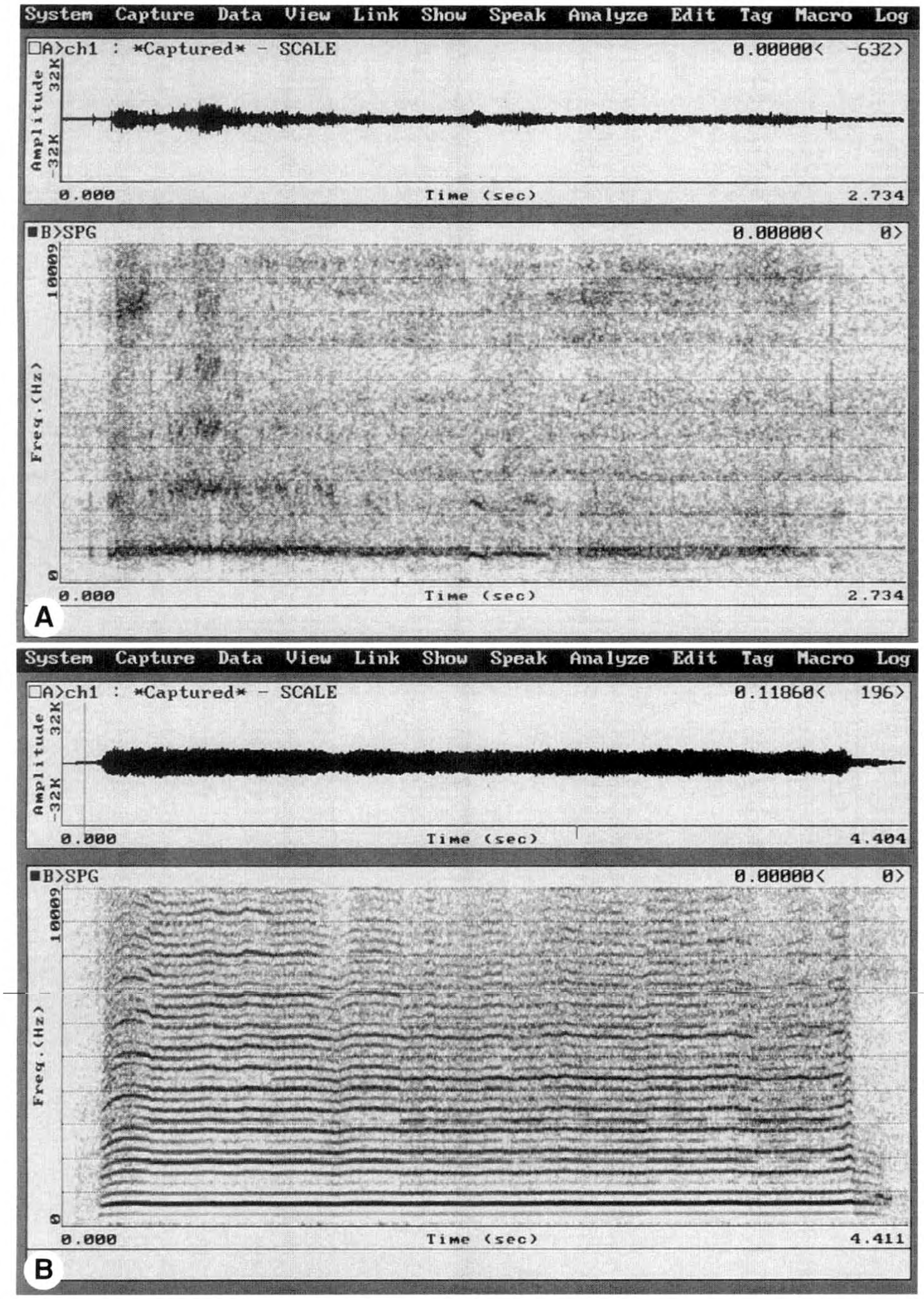

Fig 3. Sound spectrograms (narrowband Sonagrams) before and after operation. Samples contain ambient (light source) noise, because they were taken from videotape. A) Before operation. No harmonic components (mostly noise). Patient is nearly aphonic. B) After operation. Harmonic components are well identified, and vowel sounds are stable and sustained.

been instructed in how to relax, particularly during speech, how to deep-breathe, and how to avoid coughing that might elicit tight glottal closure.

DISCUSSION

Definition and Diagnosis of Spasmodic Dysphonia. The definition and diagnosis of spasmodic dysphonia are always controversial,[36-40] because they are vague and mostly based on auditory perception of the voice, which is widely variable among patients. Further, the cause or pathological entity still remains mostly unknown.

by the following criteria: 1) the voice signs of spastic dysphonia, 2) the absence of vocal cord lesions or paralysis, 3) normal remaining peripheral speech mechanisms, and 4) the resistance of symptoms to voice therapy and psychotherapy.

The present case seems to meet the above requirements. Moreover, Deems and Sataloff[40] state that patients with muscular tension dysphonia usually respond to voice therapy alone or in combination with psychotherapy, while those with spasmodic dysphonia often do not improve significantly with voice therapy alone. Murry and Woodson[39] mention that at pres-

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to follow in evaluating patients for spasmodic dysphonia, though recent studies[41-44] provide an index of severity.

Briefly, we fail to find explicit, itemized, data-substantiated criteria to differentiate between spasmodic dysphonia and muscular tension dysphonia in the pertinent literature. That there was no response to any voice therapy or psychotherapy in this case tends to preclude muscle tension dysphonia, though a clear-cut differentiation between compensatory muscle tension dysphonia and true laryngeal dystonia is not easy. The primary diagnostic bases for spasmodic dysphonia in this case include 1) an effortful, strangled, strained voice, interrupted during speech, especially on the telephone, 2) excessively tight closure of the glottis during phonation, and 3) no effect from voice and other conservative therapies.

From the viewpoint of surgical indications for midline lateralization thyroplasty, what is most important is the finding that the dysphonia is resulting from excessively tight closure of the glottis on phonation, and that it does not respond to any voice therapy.

Previous Surgical Techniques. One of the authors (N.I.)[45,46] has reported a type of thyroplasty (variation of types II and III for relaxation and slight lateralization of the vocal fold by overlapping the incised edges) for treating adductor spasmodic dysphonia with mixed results: the best result was a normal voice obtained without any recurrence as followed up for over 6 years, and the worst was a recurrence within 3 months. Similar reports with mixed results were also made by Honjo et al[47] and Tucker.[48] In our cases no excessive lowering of the vocal pitch occurred, however, because the surgery was done while monitoring the voice under local anesthesia.

Salient Points of Midline Lateralization Thyroplasty. Here described is a new technique for widening the anterior commissure, or more direct lateralization of the anterior portion of the vocal folds than that performed by the modified type III previously reported, which can be one of the options for treating spasmodic dysphonia.

The key issues include an intraoperative decision on how wide the anterior commissure should be separated, how to maintain the anterior widening, and how to prevent mucus leakage from the airway and subsequent infection. The surgery has been quite successful in restoring a normal voice, with neither complications nor recurrence as of 1 year 5 months postoperative.

Surgical Guidelines. A midline cartilage incision should be made with caution not to cut the underlying soft tissue. The effect of lateral pull of the incised edges on the voice should first be evaluated to make a judgment as to whether further lateralization of the vocal folds by making a hole at the anterior commissure is required or not. Opening into the airway at the anterior commissure, which is indicated only when simple lateralization without making a hole is considered insufficient, should be done very carefully, to prevent injury to the anterior portion of the vocal fold.

Second, judging from the voice being produced, the widening of the anterior commissure should be set so as to be sufficient for easy phonation. Although it should be decided individually, in our case the perforation created measured 2 × 3 mm, and the distance between the incised edges of the thyroid cartilage was 4 mm.

Third, the closure of the perforation with a free composite graft or a local cartilage flap must be made watertight. As for the free composite graft used to cover the perforation, the redundant soft tissue facing inside, mostly fatty connective tissue, might have been unnecessary, judging from the postoperative laryngeal findings, though it did not substantially disturb the healing course or voice. A mucosa-cartilage-perichondrium composite graft from the septum, an auricular cartilage graft, or a local small hinged flap of perichondro-chondrium may replace the one used in this case.

Suturing the graft to the thin soft tissue remaining around the perforation was the most delicate and time-consuming part of the procedure. The sutures should not enter the airway and should not catch so much tissue in the anterior portion of the vocal fold as to cause deformity (depression) of the free margin of the vocal fold, and yet the fixation must be watertight and firm enough to secure the graft contact with the recipient tissues.

Fourth, the incised cartilage edges, 4 mm apart, should be fixed in separation securely; we used a silicone shim in our case. A miniplate with screws may be an alternative, but in our hands, the silicone shim seemed safer and easier to use, because we have never had any trouble with a silicone shim in laryngoplasties. The shim had a central projecting part, 4 mm in width and 2 to 4 mm in depth (depending on the thickness of the incised cartilage), so as to just fit in the cartilage defect. This projection should not be made too high, in order to prevent forceful compression on the underlying thin soft tissue and a subsequent break into the airway.

Finally, the muscle flap covers the graft in direct contact for better viability and is interposed between the perforation site and the silicone shim in order to prevent any possible contamination from a mucus leak. This procedure is recommended to secure an uneventful postoperative course. The muscle tissue is known to be resistant to infection. The muscle flap alone, without a free graft, may be enough to close the perforation by firm fixation sutures.

Advantages of Technique. The current treatment of choice for spasmodic dysphonia is, without doubt, botulinum toxin injection. The reason we selected this new surgical treatment for the patient was because we believed it would provide a longer-lasting result without adverse effects such as temporary breathiness or hoarseness or temporary aspiration, which are often reported for botulinum toxin injection. Also, it was a modification of framework surgery that we felt comfortable performing on the basis of our extensive experience with related procedures.

Obviously, it is too early to conclude from 1 case, at this time 1 year 5 months postoperative, that this surgery can ensure permanent symptom control in adductor spasmodic dysphonia. Numerous reports have been published showing some degree of recurrence after whatever therapy was employed. However, it seems rather unlikely that recurrence will occur soon, because there is no problem such as neural regrowth, and the change in the glottal condition appears stable and permanent except for the minimal factor of scar contracture — in contrast to the previous treatments employed. The width of separation set by the operation is therefore critically important.

Above all, the surgery does not deprive the patient of the function of either the nerve or the muscle. Should a recurrence occur, revision seems much simpler and easier to perform than with the previous treatments — just replace the shim with a wider one.

The procedure seems quite beneficial to the patient in terms of time and cost. It remains unknown whether any surgery can treat abductor or tremor-type spasmodic dysphonia. The basic concept for the surgery has briefly been described elsewhere.[49] Using the same technique, we have since treated 2 other spasmodic dysphonia patients, successfully and without recurrence, and these cases will be reported elsewhere.

CONCLUSION

Midline lateralization thyroplasty, or a widening of the anterior commissure, was employed to treat a patient with adductor spasmodic dysphonia. The widening was surgically accomplished by a midline incision of the thyroid cartilage and creating a tiny perforation at the anterior commissure, which was followed by closure of the perforation with a free composite graft and by separation of the cartilage edges secured by inserting a silicone shim.

As of 1 year 5 months postoperative, the voice has been restored to normal without any sign of recurrence. The main surgical points include 1) correct judgment of the extent of the widening required, 2) no injury to the anterior portion of the vocal fold, 3) sealing of the perforation with a composite graft and a rotated muscle flap, and 4) stable separation of the incised edges of the thyroid cartilage. The results seem promising.

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EFFECTS OF BOTULINUM TOXIN ON PATHOPHYSIOLOGY IN SPASMODIC DYSPHONIA

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To determine the mechanism of symptom relief with treatment by botulinum toxin injection in persons with adductor spasmodic dysphonia (ADSD), we evaluated the effects of unilateral thyroarytenoid muscle injections on both injected and noninjected muscles in 10 subjects with ADSD, using electromyography on both sides of the larynx before and after treatment. The subjects' speech symptoms were reduced ($p = .005$) 2 weeks following injection, when the electromyographic study occurred. Muscle activation levels and the numbers of spasmodic muscle bursts decreased significantly ($p \leq .03$) postinjection in both the injected and noninjected muscles. The reductions in laryngeal muscle bursts correlated with symptom reduction ($r \geq .7$) in all muscles. Reductions in laryngeal muscle bursts did not relate to either absolute or normalized levels of muscle activity before or after botulinum toxin injection. The results suggest that changes in the central pathophysiology are responsible for changes in speech symptoms following treatment.

KEY WORDS — Botox, botulinum toxin, electromyography, laryngology, larynx, spasmodic dysphonia, voice.

INTRODUCTION

Adductor spasmodic dysphonia (ADSD) is a focal dystonia affecting the larynx during speech.[1-3] It is characterized by a strained speech quality with pitch and phonatory breaks coincident with muscle spasms during vowels in connected speech.[4] These muscle spasms are bursts of increased muscle activity, primarily in the thyroarytenoid (TA) muscles, although some burst activity has been found in the cricothyroid (CT) muscles as well.[5] The treatment of choice for ADSD is administration of type A botulinum toxin into the adductor muscles of the larynx.[6,7] Botulinum toxin A cleaves SNAP 25, a transmembrane docking protein, and blocks the release of acetylcholine into the neuromuscular junction, effecting muscle fiber denervation.[7] One of 2 botulinum toxin dosing regimens is commonly employed: either a large unilateral TA dose (approximately 8 to 15 mouse units) or a smaller bilateral TA dose (approximately 1 to 2.5 mouse units) in each TA muscle.[8] Recent studies have suggested that the unilateral dosing regimen may produce a longer duration of benefit and a shorter duration of side effects compared to bilateral injections.[8-10] Botulinum toxin results in a near-complete resolution of symptoms in ADSD in over 90% of patients from the period beginning 2 weeks after the injection and lasting until approximately 4 months postinjection, when reinnervation occurs.[11] At the conclusion of the response period, new end plates are formed by nerve fiber sprouting, and the patient's voice symptoms return.[12-15]

The pathophysiology of ADSD is not understood.[16] The adductor muscle spasms are thought to be of central origin, and their control by unilateral nerve resection or botulinum toxin may be due to partial denervation of the adductor muscles in the larynx that reduces the ability of adductor muscle spasms to interfere with voicing.[3,17] A few studies have suggested that such symptom control might be due to changes in the pathophysiology of the disorder. A case study demonstrated that a temporary unilateral chemical nerve block reduced the number of muscle spasms on the opposite side of the larynx.[4] Further, a preliminary study reported on the physiological effects of botulinum toxin injection on laryngeal muscle activation patterns in 5 patients with ADSD.[18] Four received benefit, and 1 did not. Three received unilateral injections, and 2 received bilateral injections. When the percent increase in muscle activity over resting levels was examined, decreases were found in the injected and noninjected TA muscles, but not in the CT muscles. However, the maximum activity level of each muscle was not measured, so that it was not known whether the muscle activity was reduced in amplitude, possibly due to diffusion of botulinum toxin to neighboring laryngeal muscles, reducing activation levels. Changes in spasmodic activity in other laryngeal muscles might be due to systemic effects of botulinum toxin on other muscles in the larynx.[19,20] Distant effects have been found on other muscles in the body following large-dosage injections for torticollis.[21]

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A similar study was conducted in 4 patients with oral mandibular dystonia,[22] in which the levels of activation of various jaw muscles were measured during maximum gestures, and the percentage of maximum activity was computed during speech before and after botulinum toxin injection. In these patients, only the injected muscles decreased in activity; no changes were found in the noninjected muscles. Because jaw muscles are larger and further apart than the laryngeal muscles, perhaps toxin diffusion would be less likely to occur. Another difference between the two studies, however, was in the methods of measurement of muscle activity. In the preliminary study of 5 spasmodic dysphonia patients receiving botulinum toxin injection,[18] muscle activity was measured as a percent increase over minimum resting levels. In oral mandibular dystonia,[22] activity levels were measured as a percentage of maximum levels found during isometric tasks.

Differences in the pathophysiology of the two disorders might also account for different changes in pathophysiology following treatment with botulinum toxin. In a study of torticollis patients, activity in the noninjected muscles increased following botulinum toxin injection, and this finding suggests that central patterning changes occurred following treatment with botulinum toxin.[23] Thus, different changes in pathophysiology found following botulinum toxin injection in different focal dystonias may reflect either different degrees of diffusion from injected to noninjected muscles, differences among measurement techniques, or differences in the pathophysiology of these disorders.

Recent basic studies have demonstrated central changes in motoneuron firing as a result of intramuscular injection with botulinum toxin.[24,25] Botulinum toxin type A injections into the lateral rectus muscles in cats demonstrated a loss of synapses to the motoneurons and a reduction in inhibitory and excitatory firing of these motoneurons.[24,25] Thus, changes in motoneuron firing could contribute to changes in the pathophysiology of spasmodic dysphonia, rather than the beneficial effect being solely due to decreased muscle fiber activation as a result of a chemical block of acetylcholine exocytosis.

The efficacy of botulinum toxin could also be due to altered central motoneuron pool firing as a result of changes produced in the peripheral fusimotor system. Botulinum toxin is not a selective agent within an injected muscle, and it affects both the fusimotor fibers and the motor fascicles.[26] Thus, the central effects could be due to a reduction in 1a afferent firing that alters motoneuron activity in the brain stem. The physiological effects of laryngeal muscle spindles have yet to be demonstrated in the larynx. Laryngeal adductor reflexes, however, have been demonstrated in response to stimulation of joint and tendon mechanoreceptors in the larynx.[27-29] The fibers for such mechanoreceptors are thought to be contained in the recurrent laryngeal nerve and might be affected by recurrent nerve block or sectioning, interventions effective in reducing speech symptoms in spasmodic dysphonia.[4,30] A partial weakening of the laryngeal adductor muscles as a result of botulinum toxin injection might reduce feedback from the joint and tendon mechanoreceptors, thus reducing adductor muscle activity. Symptom control of ADSD, therefore, may be produced both by changes in muscle fiber firing as a result of peripheral denervation by botulinum toxin and by changes in the central nervous system physiology.

Our purpose in this study was to examine changes in laryngeal muscle physiology in relation to symptom relief in patients with ADSD following a unilateral TA botulinum toxin injection. If the sole mechanism of symptom control by botulinum toxin injection in ADSD is via peripheral denervation, one should see activity level and muscle bursting changes only in the injected muscles. Such changes should correlate well with changes in symptom reduction only for the injected muscles. However, if activity level changes are found in both the injected and noninjected muscles of the larynx, secondary central changes are suggested. In such instances, changes in symptom production may relate to changes in muscle activity in both injected and noninjected muscles. Finally, to examine whether diffusion of the botulinum toxin to adjacent muscles is related to changes in muscle activity and spasmodic bursts, the total injection dosage, which has been shown to relate to diffusion to adjacent muscles,[19,31] was related to changes in muscle characteristics with treatment.

METHODS

Subjects. Five men and 5 women (Table 1) with ADSD were enrolled for study. The procedures were reviewed and approved by the Institutional Review Board as part of protocol number 85-N-195. None of these subjects had previously been treated with botulinum toxin. The subjects had no other signs of neurologic or speech disorders and received a diagnosis of ADSD based upon findings from a flexible fiberoptic examination performed during various speech and phonatory tasks. Criteria for diagnosis included a strained-strangled vocal quality with glottal stops occurring during vowel productions in connected speech. The voice interruptions were associ-

TABLE 1. DEMOGRAPHICS OF STUDY POPULATION

Subject	*Sex*	*Age at Disease Onset (y)*	*Duration of Disease Prior to Botulinum Toxin (y)*
1	M	57	6
2	F	42	1
3	M	28	2
4	F	19	3
5	M	40	4
6	F	32	3
7	M	40	2
8	F	38	8
9	M	42	5
10	F	36	6

TABLE 2. DOSING REGIMENS FOR TREATMENT GROUP

Patient	*Muscle Injected*	*Injections per Series*	*Total Dose (units)*
1	RTA	2	33.75
2	LTA	1	15
3	RTA	1	15
4	LTA	5	87
5	RTA	1	15
6	LTA	1	15
7	LTA	1	15
8	LTA	1	15
9	LTA	1	10
10	LTA	2	35

RTA — right thyroarytenoid, LTA — left thyroarytenoid.

ated with overadduction of the vocal folds observed on fiberoptic laryngoscopy. Three subjects received botulinum toxin injections in the right TA muscle, and 7 received injections in the left (Table 2). Some subjects required more than 1 injection to obtain symptom control. All injections were in the same TA muscle. The percutaneous approach to injection of the TA muscle is confined to 1 side of the larynx; diffusion may occur into other laryngeal muscles on the same side, such as the lateral cricoarytenoid (LCA) muscle.[32] However, because the TA muscles on opposite sides of the larynx are not contiguous, diffusion would not occur to the TA muscle on the opposite side.

Speech Symptom Recording and Measurement. Speech was tape-recorded at a constant 2-inch mouth-to-microphone distance before each subject's first injection with botulinum toxin and within 2 weeks following successful treatment, during the same clinical visit as the electromyography (EMG) posttreatment study. Subjects listened to a prerecorded instruction tape that presented several tasks to be repeated. Five were sentences: "We mow our lawn all year," "A dog dug a new bone," "The puppy bit the tape," "Did he go to the right or to the left?" and "When he comes home we'll feed him." An extended vowel production for 5 to 7 seconds was also recorded. The speech samples made before and after treatment were randomly ordered and dubbed onto a digital audiotape recorder for listener rating. No identification was provided as to whether a sample was made before or after treatment. A speech rating scale was used for measuring symptom severity. Three voice factors were rated ("overpressure," "tremor," and "voice quality/roughness") on the same 4-point scale: "0" was within normal limits; "1" was a mild and occasional deviation from normal that would be intermittently noticeable to others; "2" was a moderate deviation from normal that would detract from listener skills and disrupt speech at least once per sentence; and "3" was a severe deviation impairing speech intelligibility for communication. In addition, the number of noticeable voice breaks in sentences were counted. To derive a single measure of the degree of speech impairment, the average rating for each voice quality was multiplied by 5 (for the 5 sentences) and added to the total numbers of voice breaks counted from all 5 sentence productions.

Electromyographic Recording and Quantification Procedures. All subjects received simultaneous multichannel EMG before and after unilateral TA muscle botulinum toxin injection. The pre–botulinum toxin EMG examinations were performed at least 1 day prior to botulinum toxin injection, and a post–botulinum toxin EMG was performed between 12 and 39 days after botulinum toxin injection. The multichannel EMG recordings were performed with either concentric needle electrodes or with hooked-wire electrodes. The same electrode type was used in both the pretreatment and posttreatment studies in each patient. All subjects demonstrated an improvement in vocal quality at the time of the post–botulinum toxin EMG. Prior to each EMG recording, calibration signals measuring 2 V peak-to-peak were played from the DISA multichannel EMG unit and recorded on a Vetter reel-to-reel recorder.

The EMG recording technique has been described in detail in previous studies.[5,33] Briefly, the subject was placed supine in a fully reclining chair. The subject's neck was extended, and palpation was performed to identify the superficial landmarks (cricoid cartilage, thyroid notch, midline, and lateral extent of the thyroid cartilage). Following an intradermal injection of 1% Xylocaine with 1:100,000 epinephrine, a concentric needle electrode was placed into each laryngeal muscle by a percutaneous route. The needle electrode placement was adjustable and allowed for an accurate near-field recording of the laryngeal muscle being sampled. In some of the sub-

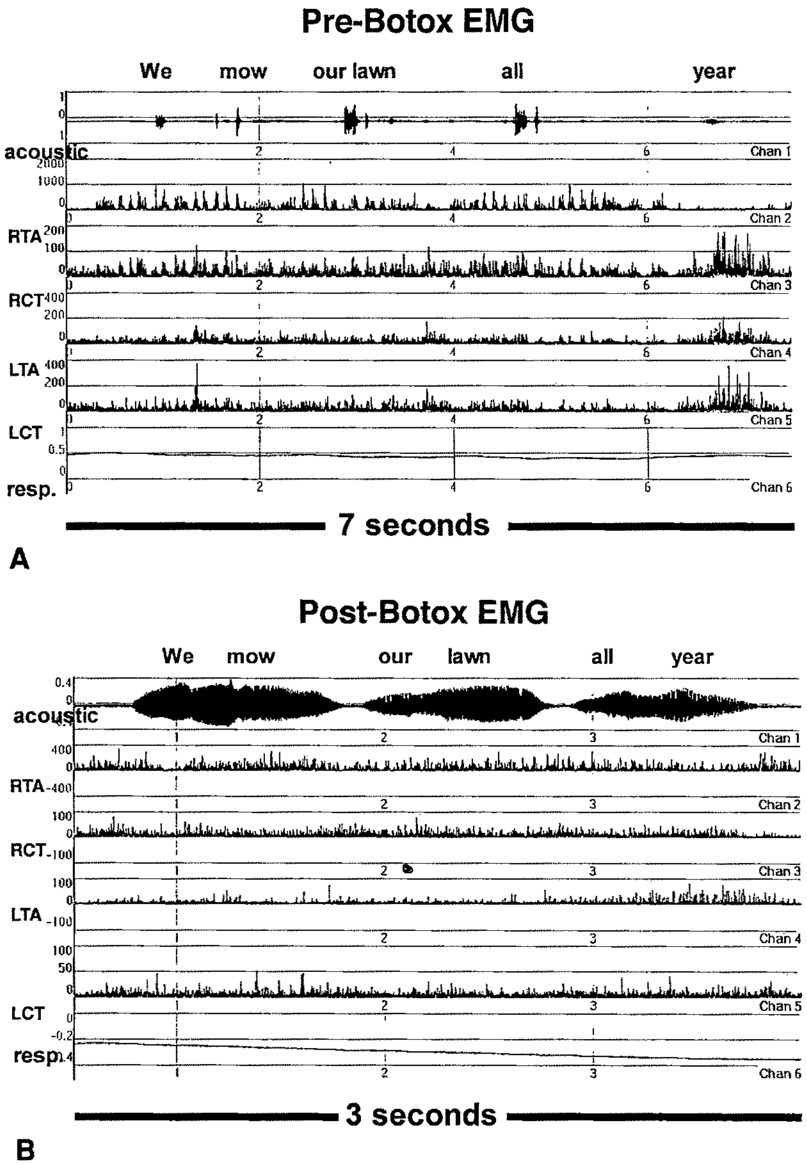

Fig 1. Multichannel A) pre–botulinum toxin injection and B) post–botulinum toxin injection electromyographic recordings for right thyroarytenoid (RTA), left thyroarytenoid (LTA), right cricothyroid (RCT), and left cricothyroid (LCT) muscles during production of sentence "We mow our lawn all year."

jects, the concentric needles were replaced with hooked-wire electrodes. The concentric needles or hooked-wire electrodes maintained a stable location in the larynx for the duration of a 1-hour study.

The muscles that were recorded included bilateral TA and CT muscles. The insertion point for the TA muscle was immediately superior to the superior cricoid cartilage border, approximately 5 mm lateral to midline, with the needle passed in a 45° superior direction and a 30° lateral direction. Verification for TA muscle insertion included increased activation during phonation, effort closure, and swallowing. Absence of phonatory bursts at voicing onset and offset confirmed lack of contamination from the LCA muscle. The insertion point for the CT muscle was at the superior border of the cricoid cartilage, approximately 15 mm lateral to midline, with the needle passed perpendicular to the cricoid cartilage so as to penetrate the inferior aspect of the extralaryngeal portion of the CT muscle. This insertion technique for the CT electrode diminished contamination from the intralaryngeal TA muscle. Verification for CT muscle insertion included increased activation with increased pitch, and an absence of increased recruitment on head elevation and swallowing confirmed lack of contamination from the nearby strap muscles or the TA muscle.

Prior to initiation of EMG recordings, inductive plethysmography (Respitrace) bands were placed around the chest and abdomen, and a Sony head-held microphone was placed. After all 4 electrode pairs were placed, simultaneous recordings of the EMG electrode, plethysmography, and microphone signals were amplified and bandpass-filtered and recorded onto the Vetter recorder. The EMG signals were bandpass-filtered between 100 and 5,000 Hz and ampli-

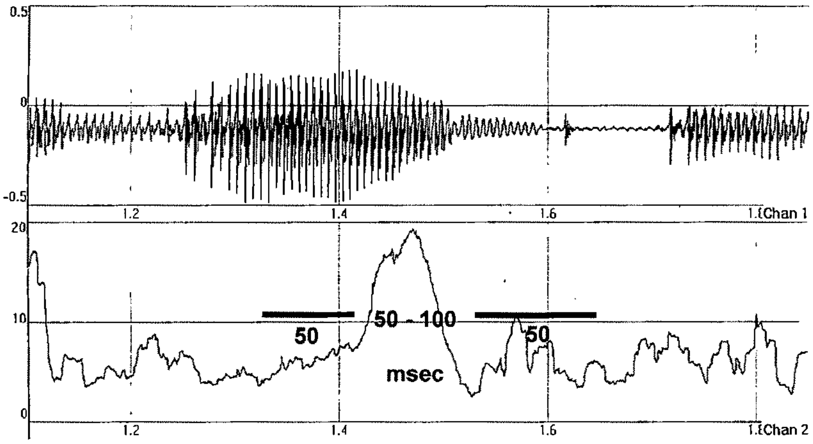

Fig 2. Illustration of process used for burst identification in 1 electromyographic recording. Electromyographic signal was rectified and smoothed with 20-millisecond sliding average. To be identified as spasmodic muscle burst, smoothed activity had to be twice amplitude of surrounding activity for 50 to 100 milliseconds. Surrounding activity was defined as average activity 50 milliseconds prior to and 50 milliseconds after burst.

fied on a DANTEC 1500 unit.

After electrode insertion, the patient was allowed to relax in the supine position for 5 minutes. After the subject relaxed, approximately 1 minute of quiet respiration was recorded. The subject was then asked to perform 3 repetitions of a series of tasks. The subject was instructed to perform speech gestures including prolonged /i/ and /i/ repetitions. Maximum gestures included a Valsalva maneuver, head raise, swallow, and high-pitched prolonged /i/. The sentences produced were "The puppy bit the tape" and "We mow our lawn all year." Examples of pretreatment and posttreatment EMG recordings are included (Fig 1).

Signal Analysis. The recorded signals were digitized off-line. Different signal types were amplified, anti-alias–filtered, and digitized at different rates. The EMG signals were low-pass–filtered at 2,000 Hz and digitized at 5,000 Hz. Speech was low-pass–filtered at 5,000 Hz and digitized at 10,000 Hz. Respiratory signals were low-pass–filtered at 200 Hz and digitized at 500 Hz. Linear interpolation from the calibration signals was used to convert the EMG signals into microvolts. To correct for the DC offset, nonrectified data were averaged for each EMG channel over 20 seconds of quiet respiration. These averages were used to correct the EMG channels for DC offset. After offset correction and rectification of the EMG signals, the minimum signal level between motor unit firings during quiet respiration was measured for each EMG channel and subtracted to remove impedance noise.

A 20-second segment of quiet respiration was selected to determine mean resting activity for each TA and CT muscle recorded. Next, all speech and nonspeech gestures were processed with an averaging 20-millisecond (ms) sliding window. The maximum of this smoothed mean activity was obtained for each muscle across all tasks to identify maximum activity achieved for each muscle recording in a subject. The mean percent of maximum activity (% Max) for the speech and prolonged vowel recordings in each muscle for each subject was processed to allow for comparisons across subjects before and after botulinum toxin injection.

Measurements were made of the numbers of muscle bursts occurring during the sentences "The puppy bit the tape" and "We mow our lawn all year." First, the EMG recordings for each of these sentences were smoothed with a sliding window computing the mean maximum activity over 20 ms. Hard copies of the EMG signals were then printed with 750-ms, nonoverlapping segments on each page. The signals were randomized, and the bursts for each sentence were counted by one of the authors. The author was blinded to the treatment status and subject name for each recording. Bursts were defined as sustained activity that was twice the surrounding activity and between 50 and 100 ms in duration. The surrounding activity was defined as the average activity occurring 50 ms prior to the burst and 50 ms after the burst (Fig 2).

Statistical Analyses. Separate, repeated multifactorial ANOVA comparisons were performed for 3 measures: maximum muscle activation level, total number of muscle bursts, and percent of maximum during speech. For each measure, the multifactorial repeated ANOVA examined the repeated treatment effect, side effects (injected versus noninjected), muscle effects (TA versus CT muscles), treatment-by-side interactions, and treatment-by–muscle type interactions. A single-factor repeated ANOVA was conducted to compare pretreatment and posttreatment speech symptom ratings. Because "pre-post" treatment effects were tested on 4 measures, a Bonferroni correction was used, yielding a criterion p value of .0125 (.05/4 = .0125).

Pearson correlation coefficients were computed to examine relationships between speech symptoms and EMG measures before treatment and relationships between changes in speech symptoms and changes

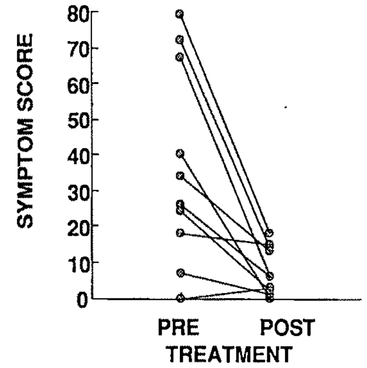

Fig 3. Speech symptom rating scores before and after treatment. Speech rating scores were derived from 4-point ratings of voice "overpressure," "tremor," and "quality" and total numbers of perceived voice breaks in 5 sentences.

in muscle activity. Finally, to examine whether injection dosage was related to muscle activity change with treatment, Pearson correlation coefficients were computed to examine relationships between percent change in EMG measures with treatment and total injection dosage.

RESULTS

Speech Symptom Changes. The speech symptom rating scores changed significantly after unilateral botulinum toxin injection on a repeated *t*-test ($t = 3.72$, $df = 9$, $p = .005$). The symptom severity rating decreased in 9 of the 10 subjects. Only the patient with the mildest ADSD symptoms increased in symptom severity after botulinum toxin injection (Fig 3).

Muscle Physiology Changes. The number of bursts counted in the EMG tracings during speech were compared with injection factors by multifactorial repeated ANOVA testing. The factors included treatment effects, side effects (injected versus noninjected), muscle effects (TA versus CT muscles), treatment-by-side interactions, and treatment-by-muscle type interactions. Significant treatment effects were demonstrated ($F = 7.5$, $df = 1$, $p = .009$). No significant ($p > .05$) differences in the numbers of breaks were found by side or by muscle. Treatment-by-side or treatment-by-muscle interactions did not occur. The reductions in number of spasmodic bursts were similar in the injected and noninjected TA and CT muscles (Fig 4).

On a multifactorial repeated ANOVA, the maximum level of muscle activity in microvolts demonstrated significant changes with treatment ($F = 8.802$, $df = 1$, $p = .005$) and no significant side, muscle, treatment-by-side, or treatment-by-muscle interactions ($p > .0125$). The maximum levels of muscle activity on isometric gestures decreased in all 4 muscles studied (Fig 5).

The individual mean values in percent of maximum muscle activity are plotted in Fig 6. The mean percent of maximum levels of muscle activity during sentence production and a prolonged vowel showed a similar treatment effect ($F = 11.199$, $df = 1$, $p = .002$). No significant muscle, side, muscle-by-treatment, or side-by-treatment interactions were

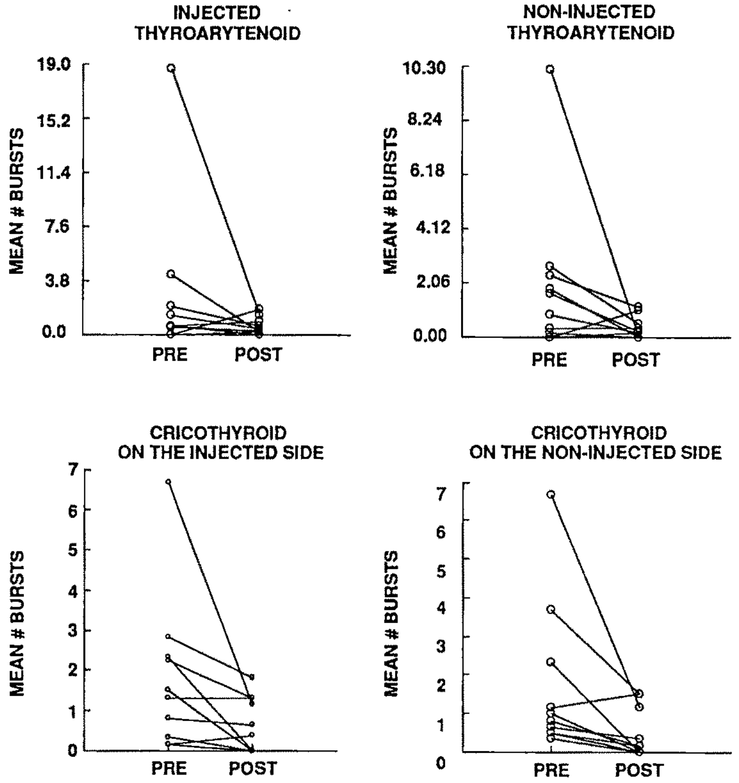

Fig 4. Mean numbers of muscle bursts in each subject counted from electromyographic recordings before and after botulinum toxin injection in each of 4 muscles.

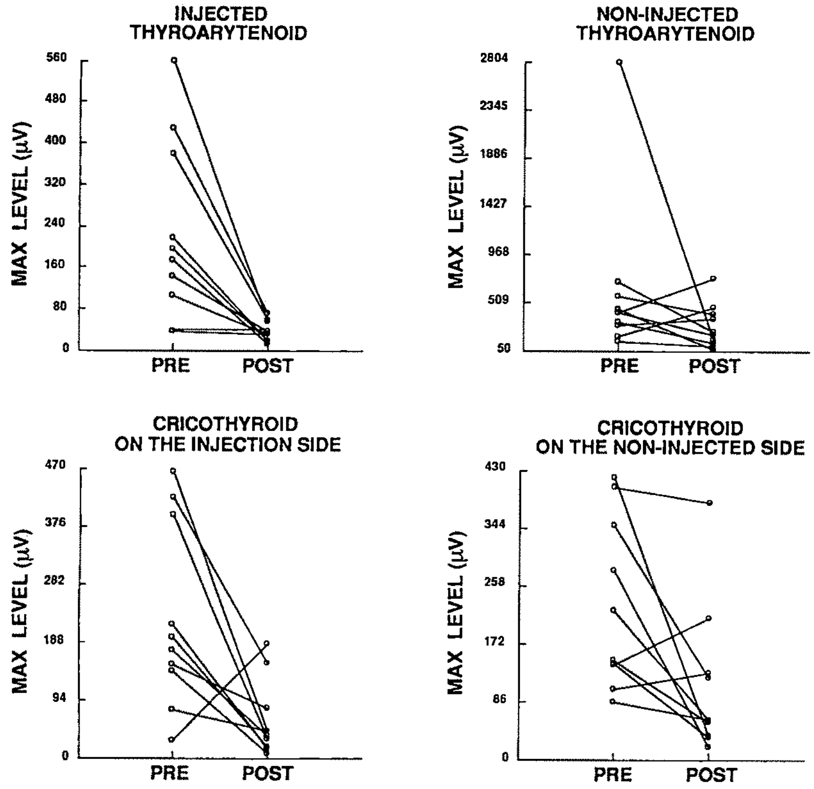

Fig 5. Maximum level of activity found for each muscle in each subject before and after injection with botulinum toxin. Maximum activity in microvolts was identified from rectified and smoothed recordings made during speech, Valsalva maneuver, head raising, swallow, and high-pitched vowel prolongation.

found (p > .0125).

Relationships Between Speech and Muscle Physiology. Pearson correlation coefficients were computed between the total number of breaks and the number of muscle bursts in each of the 4 muscles before treatment (Table 3). The number of speech breaks was related to the number of muscle bursts in all 4 muscles ($r \geq .67$, $p \leq .05$).

Similarly, following treatment, the change in the number of muscle bursts and the change in the number of speech breaks were related (Table 4). A reduction in muscle bursts was closely related to the reduction in the number of speech breaks in 3 of the 4 muscles ($r \geq .67$, $p \leq .05$), and a similar but nonsignificant trend in the CT muscle on the noninjected side was identified ($r = .65$).

Relationships Between Spasmodic Bursts and Muscle Activation Levels. No consistent set of relationships between the number of spasmodic bursts and the measure of muscle activation level was found in any of the 4 muscles studied (Table 4). Further, no consistent relationships were found between the reduction in the number of spasmodic muscle bursts and the change in muscle activation levels (either the change in maximum activation level or the change in the mean activation level during speech).

Relationships Between Spasmodic Bursts and Muscle Activation Levels With Injection Dosage. No consistent set of relationships between the measures of change in muscle activity and change in spasmodic bursts and the total dosage level was found in any of the 4 muscles studied (Table 5). Significant ($p \leq .05$) but opposing relationships were only found in the noninjected TA muscle; the percent change in mean activity during speech was greater in the patients who received larger dosages on the injected side. The opposite finding was also the case; however, the reduction in the number of muscle bursts was less in the patients receiving higher injection dosages. Thus, there was no consistent pattern of relationship between change in muscle activity and injection dosage in any of the muscles.

DISCUSSION

The purpose of this study was to better understand the mechanism of symptom control in ADSD with botulinum toxin injection. In this study, the maximum activity of both injected and noninjected TA and CT muscles decreased after a unilateral botulinum toxin injection. This was a surprising finding, given the variability of the absolute maximum electrical activity relative to small position changes of the recording electrode within the same muscle.[34] Previous studies have not shown a uniformly increased maximum activity in the TA or CT muscles in patients with ADSD compared to normals.[33,35] Thus, we would not have anticipated a decrease in what has been previously described as a normal level

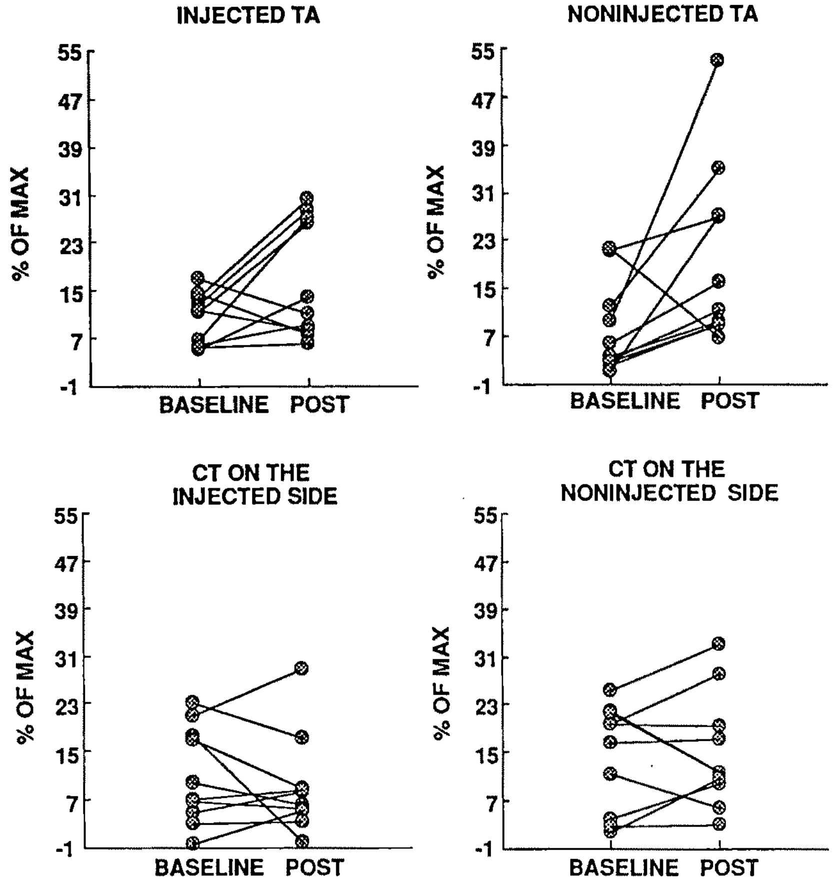

Fig 6. Mean percent of maximum activity for electromyographic recordings made during sentences and prolonged vowel for each muscle in each subject before and after botulinum toxin injection.

of maximum activity in patients with ADSD. Some may argue that these EMG findings were due to a diffusion of botulinum toxin to the noninjected muscles. Certainly, this is likely, and has been shown for the ipsilateral LCA muscle, which is adjacent to the TA muscle and can be affected when the TA is injected.[20,36] Diffusion to the contralateral CT muscle on the outside of the thyroid cartilage on the opposite side of the larynx would be very unlikely. It has been demonstrated that the fascia between muscles can reduce the spread of botulinum toxin somewhat.[19] The dosage level, which has been shown to relate to the amount of diffusion of toxin to adjacent muscles[19,31] and may relate to systemic circulation,[21] was not systematically related to the reduction in muscle activity, or more important, to the reduction in number of spasmodic bursts across muscles. In fact, the only relationship was in the opposite direction — a finding suggesting that the number of bursts in the noninjected TA muscle on the opposite side was reduced to a greater degree in patients receiving smaller injection dosages (Table 5). Neither the TA nor the CT muscles have muscle fibers communicating with the TA muscle on the opposite side of the larynx, and the anatomic distance and compartmentalization of the ipsilateral injected TA from the contralateral noninjected CT muscle on the opposite side of the larynx are considerable. Therefore, our results do not support the conclusion that the changes are the re-

TABLE 3. PEARSON CORRELATION COEFFICIENTS BETWEEN NUMBERS OF SPASMODIC MUSCLE BURSTS AND MUSCLE ACTIVATION MEASURES AND SPEECH BREAKS PRIOR TO BOTULINUM TOXIN INJECTION

Muscle	*Side*	*Maximum (μV)*	*% Max During Speech*	*No. of Speech Breaks*
Thyroarytenoid	Injected	.35	.03	.70*
	Noninjected	.07	−.43	.69*
Cricothyroid	Injected	.42	−.44	.70*
	Noninjected	.07	−.43	.69*

% Max — mean percent of maximum muscle activity.
*Statistically significant.

TABLE 4. PEARSON CORRELATION COEFFICIENTS BETWEEN CHANGES IN NUMBERS OF SPASMODIC MUSCLE BURSTS AND CHANGES IN MEASURES OF MUSCLE ACTIVATION AND CHANGES IN SPEECH BREAKS WITH BOTULINUM TOXIN INJECTION

Muscle	*Side*	*Maximum (μV)*	*% Max During Speech*	*No. of Speech Breaks*
Thyroarytenoid	Injected	−.11	−.22	.76*
	Noninjected	−.11	.49	.76*
Cricothyroid	Injected	.29	−.12	.73*
	Noninjected	−.52	.25	.65

*Statistically significant.

TABLE 5. PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL DOSAGE AND PERCENT DECREASE IN MEASURES OF MUSCLE ACTIVATION AND CHANGES IN NUMBERS OF SPASMODIC MUSCLE BURSTS WITH BOTULINUM TOXIN INJECTION

Muscle	*Side*	*% Change in Maximum (μV)*	*% Change in Mean During Speech*	*% Change in No. of Bursts*
Thyroarytenoid	Injected	−.42	−.17	.51
	Noninjected	.33	.76*	−.76*
Cricothyroid	Injected	.21	−.09	.10
	Noninjected	.16	−.57	−.04

*Statistically significant.

sult of diffusion of botulinum toxin to the other laryngeal muscles studied.

To address issues of electrode sampling error with maximum activity, we normalized the activity level for each muscle as a percent of the maximum activity recorded for that muscle. Again, a significant treatment effect was noted, with no injection or muscle type interactions. However, in all of the muscles (injected and noninjected TA muscles and both noninjected CT muscles), the percent of maximum activity increased.

As noted in Fig 4, bursting activity decreased significantly in all muscles tested. Glottal interruptions associated with TA burst activity have been identified in the past,[5] and the reduction in spasmodic bursts in all 4 muscles was related to a decrease in speech breaks. Decreases in spasmodic muscle bursts occurred in both the injected and noninjected TA muscles and both noninjected CT muscles.

The loss of bursting activity and the diminution of maximum electrical activity in all muscles tested suggest a reduction in overall motoneuron activity for both the injected and noninjected muscles. The mechanism by which this occurs is unclear. Recent studies have indicated that intracellular retrograde axonal transport of botulinum toxin in motoneurons may occur,[24,25] suggesting a possible effect of *Clostridium botulinum* toxin on the presynaptic neuron, as has been identified for the *Clostridium tetani* toxin.

Our results demonstrating a decrease in maximum activation level and overall level of muscle activity with treatment by botulinum toxin suggest a central reduction in motoneuron activity. However, the major change occurring with treatment was a significant reduction in the spasmodic bursting, which related closely to improvements in the speech symptoms. The frequency of occurrence of muscle bursts did not relate to the level of muscle activity either at rest, during speech, or on maximum activation. Therefore, the results do not suggest that a decrease in muscle activity level as a direct effect of botulinum toxin was the major factor in the reduction in muscle spasms.

Similar changes occurred in all of the laryngeal muscles studied, although injections were administered to only 1. These changes were not dependent upon a change in muscle activation level after treatment. Rather, the generalized effect across all muscles indicates that the effect of botulinum toxin on the generation of spasmodic muscle bursts affected all laryngeal muscles to a similar degree regardless of whether they were injected or not. The presence of muscle spindles in the TA muscle has been demonstrated.[37] In addition, many other mechanoreceptors are present in the larynx.[27-29,38] The physiological significance of feedback from spindle afferents and other mechanoreceptors within the larynx has not been well studied. One may hypothesize that these mechanisms may provide feedback regarding the degree of adductory force to the laryngeal motoneuron pool. Normally, such feedback might add to the level of motoneuron drive. Sensory feedback changes as a result of botulinum toxin might be an indirect effect of injection that would affect all of the muscles to a similar degree. The reduction in adductory force as a result of injection on 1 side would reduce feedback from joint mechanoreceptors during closure for speech on both sides of the larynx. Such sensorimotor feedback may have a role in the generation of muscle spasms in ADSD. If sensorimotor responses are altered in ADSD, then a reduction in afferent stimulation as a result of reductions in adductory force may result in fewer adductor reflex responses in all the muscles affected in spasmodic dysphonia. Studies of the effects of afferent stimuli on laryngeal muscle activation are needed in unaffected subjects and patients with ADSD to address these issues.

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BOTULINUM TOXIN INJECTIONS FOR ESSENTIAL VOICE TREMOR

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Fifteen patients, 13 women and 2 men, with a mean age of 72.7 years (56 to 86 years) and a clinical diagnosis of essential voice tremor, were treated with botulinum injections to the thyroarytenoid muscles, and in some cases, to the cricothyroid or thyrohyoid muscles. Evaluations were based on subjective judgments by the patients, and on perceptual and acoustic analysis of voice recordings. Subjective evaluations indicated that the treatment had a beneficial effect in 67% of the patients. Perceptual evaluations showed a significant decrease in voice tremor during connected speech ($p < .05$). Acoustic analysis showed a nearly significant decrease in the fundamental frequency variations ($p = .06$) and a significant decrease in fundamental frequency during sustained vowel phonation ($p < .01$). The results of perceptual evaluation coincided most closely with the subjective judgments. It was concluded that the treatment was successful in 50% to 65% of the patients, depending on the method of evaluation.

KEY WORDS — acoustic analysis, botulinum toxin, perceptual evaluation, voice tremor.

INTRODUCTION

Tremor can be defined as quasi-regular oscillations of a part of the body around a fixed point. A normal (physiological) tremor of the limbs usually has a frequency of 8 to 13 Hz.[1] The voice equivalent of physiological tremor during sustained phonation or singing is probably vibrato (frequency usually 5 to 7 Hz).[2-5] Movement fluctuations affecting any portion of the speech system (respiratory, phonatory, or articulatory) may create voice tremor.[2] This results in involuntary, slow sound pressure amplitude modulations and frequency modulations during phonation.[3] Voice tremor can be perceived as regular fluctuations in pitch and amplitude during sustained vowels in speech that result in a quavering or tremulous voice quality.[6]

Voice tremor is a common feature of several neurologic disorders and is considered to be a sign of neurologic disturbance. Some disorders may show voice tremor in combination with dysarthric speech, eg, Parkinson's disease, amyotrophic lateral sclerosis, or cerebellar disorders.[1,2,7,8] Tremor might be present in laryngeal dystonia. In fact, 25% to 65% of patients with spasmodic dysphonia also have voice tremor.[2,7,9,10]

Essential tremor has a reported prevalence of 0.4%. There may be a hereditary factor in up to 60% of cases, and essential tremor is possibly inherited as an autosomal dominant trait.[11-13] The most common manifestations are hand and head tremor, but voice tremor occurs in up to 20% of the cases.[12,13] The mechanism is unknown, but it has been proposed that the origin of tremor is oscillations within the cerebello-olivary pathways relayed via the thalamus and motor cortex to the spinal cord.[14,15]

Since essential voice tremor is activation-mediated, it is often most noticeable during sustained vowels. The perceptual quality of severe essential voice tremor is sometimes similar to that of spasmodic dysphonia, and these disorders are often confused by inexperienced listeners. The voice is characterized by pitch breaks and vocal arrests from adductory or abductory movements of the vocal folds during phonation.[16] Glottal airflow is interrupted or excessive. At laryngoscopy, phonatory abductory and adductory movements are often observed, not only in chest voice but also during falsetto (in contrast to adductor spasmodic dysphonia [ADSD] without tremor). In previous studies, an oscillation rate of essential voice tremor between 4 and 10 Hz has been reported, including both frequency and amplitude modulations.[6,17-19] Perturbation measures (eg, jitter or shimmer) in essential tremor have been found to be similar to[17] or slightly higher[18,20] than those in normal voices. Tremor patients suffer from strenuous, physiologically and psychologically tiring speech that often leads to social isolation similar to that experienced by patients with spasmodic dysphonia.[21] Treatment with botulinum toxin injections to the laryn-

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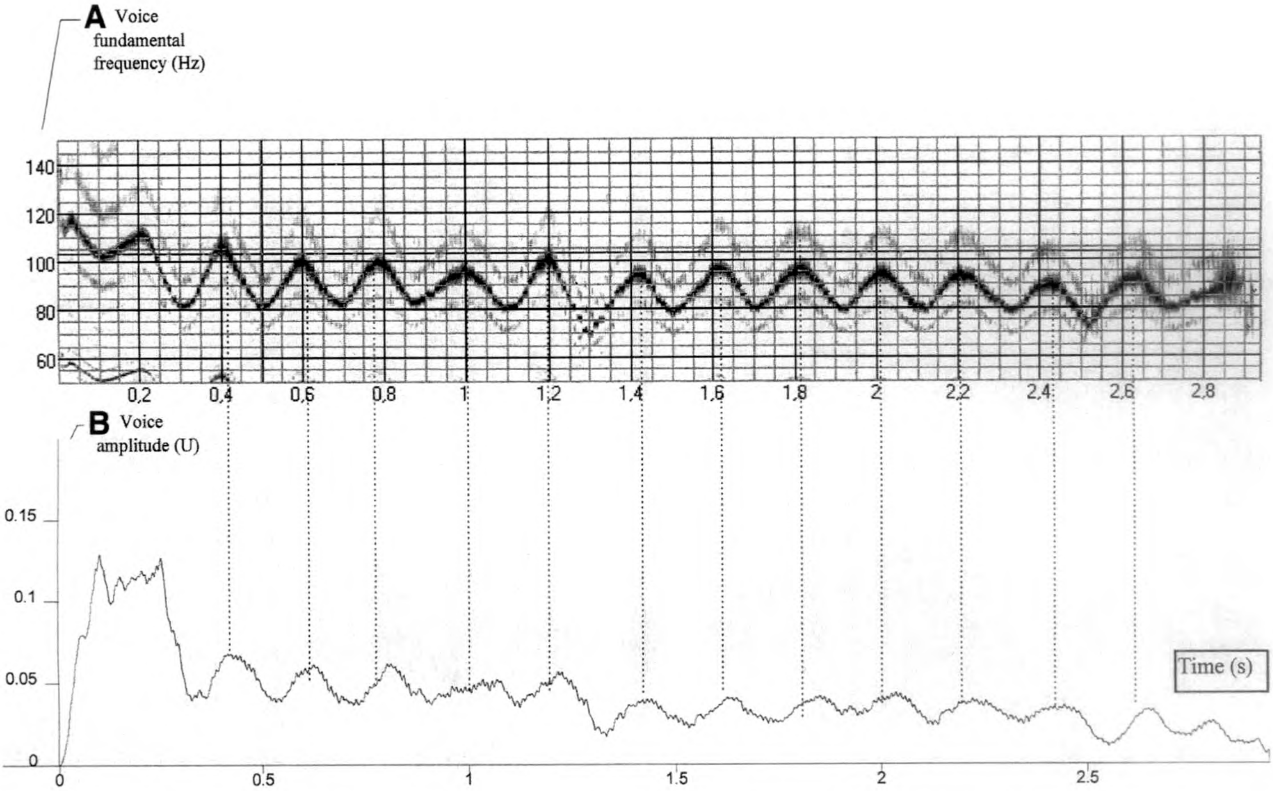

Acoustic analysis of sustained vowel /a/ for 1 patient. A) Analysis of fundamental frequency (F0) with tremor oscillations by use of "Corr" program. B) Amplitude curve analyzed with "Extract" program. Amplitude tremor oscillations are in phase with F0 oscillations.

geal muscles has been reported to be beneficial.[7]

For several years, phoniatric departments in Stockholm have treated essential voice tremor with botulinum toxin injections to the laryngeal and extralaryngeal muscles. The aim of the present study was to investigate the effects of this treatment by means of subjective evaluation and perceptual and acoustic analysis.

MATERIAL AND METHODS

Clinical Characteristics. Fifteen patients, 13 women and 2 men, were found to have essential voice tremor. Their mean age was 72.7 years (56 to 86 years). The clinical diagnosis was based on history, general and neurologic examination, laryngoscopy, and perceptual voice evaluation. No evidence of other neurologic disease was found. Six of the patients had close relatives with essential tremor, and some had voice tremor in combination with limb or head tremor. Fiberoptic laryngeal examination revealed a normal gross anatomy and normal respiratory movements of the vocal folds, although in some cases tremor movements were obvious during respiration, as well. At phonation there was more or less regular abductory and adductory tremor of the vocal folds (sometimes in combination with pharyngeal tremor). In a few patients, additional vertical tremor movements of the larynx were noted. Tremor was present during sustained modal as well as falsetto phonation, and the ordinary speech voice quality was tremulous to various degrees. Fundamental frequency (F0) and amplitude tracings showed slow modulation over time (see Figure). Some patients with severe tremor had interruptions of voice (voice breaks) during sustained phonation.

Ten of the patients had received voice training from a speech pathologist before injections. The training consisted of breath support exercises and phonation exercises intended to stabilize phonation. Five patients had between 5 and 10 sessions, and 5 patients, between 10 and 20 sessions. Five patients were offered voice training, but refused or stopped the training after only a few sessions. The voice training had little lasting benefit, and none of the patients were satisfied with only this treatment. One of the patients had experienced temporary relief of the tremor during propranolol medication.

Injections of botulinum toxin (Botox) were performed under electromyographic guidance by using a percutaneous approach and a monopolar needle for injecting the thyroarytenoid (TA) muscles.[22] Needle position was verified by typical laryngeal gestures such as swallowing and phonation. The cricothyroid

TABLE 1. PATIENT DATA AND SUMMARIZED RESULTS OF BOTULINUM TOXIN INJECTIONS FROM SUBJECTIVE, ACOUSTIC, AND PERCEPTUAL EVALUATIONS

Patient	*Sex*	*Age (y)*	*Site of Injection and Units*	*Subjective Results*	*Acoustic Results for Speech*	*Perceptual Results for Speech*	*Acoustic Results for Vowels*	*Perceptual Results for Vowels*
1	F	86	TA 1	Unchanged	Positive	Unchanged	Missing	Missing
2	M	66	TA 1.25	Positive	Positive	Unchanged	Negative	Negative
3	F	70	TA 1.25	Unchanged	Negative	Unchanged	Unchanged	Positive
4	F	80	TA 5 + CT 7.5	Unchanged	Positive	Unchanged	Negative	Negative
5	F	68	TA 1.25-2.5	Positive	Positive	Unchanged	Positive	Unchanged
6	F	77	TA 1.25	Positive	Positive	Positive	Unchanged	Positive
7	F	76	TA 1.25-2.5	Positive	Positive	Positive	Positive	Positive
8	F	81	TA 1.25-2.5	Positive	Negative	Positive	Negative	Unchanged
9	F	67	TA 1.25	Positive	Positive	Unchanged	Unchanged	Unchanged
10	F	65	TA 1.25 + TH 10	Positive	Positive	Positive	Positive	Unchanged
11	F	81	TA 2.5	Positive	Positive	Positive	Positive	Positive
12	M	56	TA 1.25	Unchanged	Positive	Unchanged	Positive	Negative
13	F	74	TA 0.6-1.25	Unchanged	Negative	Negative	Negative	Negative
14	F	66	TA 1.25	Positive	Positive	Positive	Unchanged	Unchanged
15	F	77	TA 2.5	Positive	Unchanged	Positive	Positive	Positive

TA — thyroarytenoid (vocalis) muscles injected bilaterally, CT — cricothyroid muscles, TH — thyrohyoid muscles.

(CT) and thyrohyoid (TH) muscles of a few patients were injected (Table 1). Needle position was verified with high-pitched phonation (CT) and by alternating moving the head forward and falsetto phonation (TH). Most patients were treated with repeated injections, usually at 3-month intervals.

Subjective Evaluation. Subjective evaluation consisted of each patient's subjective description of voice symptoms and side effects (such as transient hoarseness or swallowing problems) in terms of the effect of treatment. The patients were asked to rate their voice symptoms at follow-up as compared to the status before the injections using the categories positive, unchanged, or negative. This was noted in their medical records.

Voice Recordings and Perceptual Evaluation. Recordings were made on digital audiotape before and about 1 month after the latest injection. Each reading contained a standard Swedish text of about a 45-second duration and the sustained vowels /a/, /æ/, /e/, and /i/ at the most comfortable and stable pitch and intensity. The duration of the vowels varied from 0.7 second to about 5 seconds. The shorter vowels were produced by patients unable to sustain longer tones due to the severity of their tremor.

Perceptual evaluation was performed more than 6 months after voice recordings. A continuous 100-mm visual analog scale[23] was used for evaluation of both the connected speech and the sustained vowels. The scale was constructed similar to visual analog scales used for ratings of pain and other symptoms. The 2 parameters rated were degree of voice tremor and occurrence of voice breaks (phonation stops within the vowel). Two phoniatricians (authors S.H. and P.-A.L.) concurred when assigning the ratings. A total of 30 randomly mixed samples of connected speech recorded before and after treatment were evaluated. Frontal and back vowels of the longest duration were selected from each sample. The same vowels were analyzed before and after injection. One patient could only produce very short vowel segments, and therefore only running speech was analyzed. Another patient's back vowel segments were too short, and only frontal vowels could be analyzed. Thus, a total of 55 vowels were evaluated.

Consistency among the evaluators was tested for 8 duplicates of connected speech and 8 vowels (27% and 15% of the samples, respectively). The correlation between repeated ratings was high for the connected speech parameters (r = .97). The correlation for repeated vowel samples was also high (r = .92 for the tremor parameter and r = .75 for the voice break parameter).

Acoustic Analysis. The recordings were digitally fed into a personal computer with down-sampling to 16 kHz. For analysis of connected speech samples, the Swell program was used.[24] The F0 time-series curves were extracted with a double peak-picking algorithm. The raw F0 contour was further low-passed at 20 Hz to produce an F0 trend curve that depicted slow frequency variations more clearly. The standard deviation of this F0 trend (F0 SD) was calculated as a measurement of the tremor magnitude for the voices in the study.

For analysis of sustained vowels, 2 programs were

TABLE 2. PERCEPTUAL AND ACOUSTIC PARAMETERS MEASURED BEFORE AND AFTER BOTULINUM TOXIN INJECTIONS IN 15 PATIENTS

	Speech						*Vowels*											
	Tremor (mm VAS)		*Voice Breaks (mm VAS)*		*F0 SD (Hz)*		*Tremor (mm VAS)*		*Voice Breaks (mm VAS)*		*Tremor Frequency (Hz)*		*Max Tremor Extent (semitones)*		*F0 SD (Hz)*		*Amplitude SD (arb u)*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Mean	61	47	32.3	21.5	36.7	33.7	50	53	30	26.5	4.6	4.6	4.8	6.2	21.5	24.2	4.1	4.5
		*		NS		NS		NS		NS		NS		NS		NS		NS
SD	21.1	22.4	35.6	29.1	13.3	12.2	22	25	31	30	0.8	0.5	3.1	3.2	15.3	17.1	2.0	1.8
Min	20	10	0	0	10.7	9.8	20	0	1	0	3.2	3.8	1.6	3.5	6.6	7.7	1.6	2
Max	95	85	95	75	63	54.6	90	92	90	95	6.2	5.4	10.3	14.5	83.8	71.4	10	9.7

Perceptual evaluation by use of 100-mm visual analog scale (VAS).[23] F0 and amplitude standard deviations were used as measurements of tremor variation (F0 SD, amplitude SD).

*$p \leq .05$ on paired *t*-test for parameter before and after injection. arb u — arbitrary units; NS — no significant difference on *t*-test.

used. In the "Corr" program, the autocorrelation function was used to match 2 signal time frames as a function of the delay between them.[25] The most probable fundamental curve got the highest density on a time-frequency plot (see Figure). Tremor frequency (Trem freq) was calculated. The variation of tremor frequency was estimated as the standard deviation around the mean F0 (F0 SD). The maximum tremor extent (peak-to-peak) was also calculated (Trem ext).

The "Extract" program was used for obtaining an amplitude time-series curve for the acoustic signal.[26] In this program, the signal was rectified and low-pass filtered at zero phase. In order to analyze slow amplitude (sound pressure) variations, the low-pass filter was set at 20 Hz (see Figure). The magnitude of amplitude tremor was estimated as the standard deviation of the extracted amplitude curve (Amp SD).

RESULTS

The results of treatment effects by patient subjective evaluation are presented as a part of Table 1. Ten of 15 (67%) patients had a positive effect. The remaining patients had no benefit or suffered from side effects to such an extent that they discontinued treatment. Most patients (12 of 15) had a temporarily breathy and weak voice for 1 to 2 weeks. In 3 cases, the hoarseness lasted up to 4 weeks. Three patients also specifically noted slight swallowing difficulties after injections.

Perceptual Evaluation and Acoustic Analysis. The results of perceptual evaluation and acoustic analysis are summarized in Tables 1 and 2.[23]

The mean values of the perceptual evaluation of connected speech samples showed a significant decrease of the degree of tremor after injection: from a mean of 61 mm before to 47 mm after injection on the 100-mm visual analog scale ($p = .015$ at paired *t*-test). Neither the voice breaks during connected speech nor the tremor or voice breaks during the sustained vowels showed any significant changes after treatment.

Acoustic analysis of connected speech showed a nearly significant decrease for the F0 variations (F0 SD) after treatment ($p = .06$). The F0 did not change significantly. For the sustained vowels, there was a significant decrease in F0 after treatment ($p < .01$). Mean frequency of tremor (Trem freq), initially 4.6 Hz (SD 0.8), was unchanged after treatment. Tremor extent was initially 4.8 semitones (1.6 to 10.3) and did not change significantly. The other 2 parameters for tremor magnitude (Trem SD and Amp SD) showed no significant changes.

Summary of Results. Table 1 summarizes the patients' subjective opinions about treatment, acoustic analysis, and perceptual ratings. If a patient's perceptual ratings were lower after treatment for both parameters (tremor and voice breaks), the voice result was labeled as positive. If the perceptual ratings were higher, the voice result was labeled as negative. If only 1 parameter changed, a magnitude of ±40 mm was required for a positive or negative label. Other ratings with no clear pattern were regarded as unchanged.

For acoustic analysis of connected speech before and after treatment, the F0 SD parameter was studied for each patient. A decreased value was interpreted as a positive treatment result, and vice versa. In order to summarize the acoustic analysis of vowels, the results for the parameters Trem ext, F0 SD, and Amp SD were compared. If at least 2 of 3 parameters decreased, the treatment result was labeled as positive, and vice versa. Other results were labeled as unchanged.

As shown in Table 1, 7 patients (47%) with a subjectively positive treatment result also had positive results from at least 2 other evaluation methods. Two patients had no treatment effect when evaluated subjectively or by the other procedures.

DISCUSSION

Although most patients with essential voice tremor are older than 65 years, some patients suffer from pronounced symptoms at earlier ages (before retirement). The social consequences of restricted communication ability are often severe, resulting in isolation. There are few effective treatment options. Voice therapy usually has little effect, and pharmacotherapy, including propranolol and primidone, has shown mixed results.[13,19,27-29] The results of the present study corroborate previous findings that botulinum toxin injections in laryngeal muscles may be effective for diminishing symptoms. Injection treatment with our present program was effective for 50% to 60% of the patients, depending on the method chosen for evaluation. Thus, it is considerably less efficient than botulinum toxin injections for ADSD. From 80% to 100% of patients with ADSD show a positive effect, with a normal or close-to-normal voice.[21,30] There may be several reasons for this difference in results. The pathophysiological mechanism of voice tremor is most probably different from that of spasmodic dysphonia. In tremor, cerebello-olivary systems are believed to be involved, often affecting several laryngeal muscles, as well as pharyngeal and respiratory muscles.[31,32]

At present, we are injecting laryngeal (or extralaryngeal) muscles only. In an electromyographic study by Koda and Ludlow,[32] the vocalis muscle (TA) showed a high extent of tremor involvement, and the CT and external laryngeal muscles were often involved as well. In that study, only 2 of 8 patients showed tremor activity of the respiratory apparatus. Our results from injecting primarily the TA muscles, however, support the findings from Koda and Ludlow. Ideally, a diagnostic procedure would include analysis of all these subsystems (including fiberscopic laryngeal examination, acoustic analysis, electromyography, and respiratory evaluation) for prognostic purposes and for tailoring an injection strategy.

In the present study, the patients treated with slightly higher doses (2.5 U bilaterally injected into the TA muscles) tended to have a better effect. In 1 case with evident vertical laryngeal tremor movements, injection of extralaryngeal muscles was beneficial.

Our results (Table 1) indicate that perceptual evaluation of voice tremor was the most reliable treatment evaluation, as it corresponded fairly well to each patient's subjective opinion about the results. We did not attempt to quantify observations from fiberoptic laryngeal examination or electromyography findings.

We expected perceptual and acoustic analysis of sustained vowel phonation to be more sensitive than that of connected speech for tremor evaluation. This was not verified by the present study. Of the acoustic methods, the standard deviation of fundamental frequency (F_0 SD) during speech showed a nearly significant decrease after treatment ($p = .06$), whereas the perceptual degree of tremor during speech decreased significantly ($p = .015$). The F_0 SD, however, is probably sensitive to other voice and speech characteristics besides tremor (eg, voice instability, speech intonation, prosody), and this sensitivity may limit the usefulness of the parameter for tremor evaluation. The F_0 during sustained vowels decreased nearly significantly, but almost all vowels were in the normal speaking ranges for males and females, respectively.[33] However, since the TA muscle is known to be active during a pitch increase (especially in modal register phonation), the decrease in F_0 might be an effect of the botulinum toxin injected.[3] Further studies are needed to clarify which acoustic parameters are most useful for tremor evaluation.

In previous investigations, differences between voice vibrato and tremor have been studied. The average vibrato rate during sustained phonations varies between 4.5 and 7 Hz, with a standard deviation of less than 0.5 Hz and a maximal extent (peak-to-peak) of 1.4 semitones.[5,18,34,35] Titze et al[36] found a natural vocal fold oscillating frequency (vibrato) of 5 Hz for a human subject by stimulating the CT muscle artificially. In the present study, tremor rates before treatment varied between 3.2 and 6 Hz, as compared to those found by Koda and Ludlow[32] (4.7 to 5.2 Hz). Hence, the frequency component of essential tremor seems to be slightly lower than that of vibrato. Moreover, the standard deviation of tremor rate before treatment found in our study was 0.8 Hz, and the maximal extent was 4.8 semitones. Thus, frequency variations of tremor are larger than those of vibrato, and the maximal extent is larger. One may speculate that disordered cerebellar control of voice F_0 results in undamped voice source oscillations during tremor as compared to vibrato. If so, tremor oscillations of the voice source may finally become totally irregular, resulting in phonation arrests. This is indeed the case for patients with severe tremor when phonating on sustained vowels. Further studies of vocal fold oscillation patterns with high-speed imaging equipment and acoustic analysis are needed to substantiate this observation.

CONCLUSIONS

1. Botulinum toxin injections were beneficial to 50% to 65% of the patients with essential voice trem-

or in the study.

2. The effects could be verified with both perceptual and acoustic voice analyses, ie, the perceptual degree of tremor and the standard deviation of fundamental frequency.

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MUCOCILIARY TRANSPORT PATHWAY ON LARYNGOTRACHEAL TRACT AND STENTED GLOTTIS IN GUINEA PIGS

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We investigated the laryngotracheal mucociliary transport pathway of guinea pigs in vivo and immediately postmortem. Only intraperitoneal anesthesia was used during the procedure to avoid the disturbance of mucociliary function. Resin particles were used as the marking substance. A microcolpohysteroscope was placed at different levels in the laryngotracheal region for observing the marking particles and recording the transport pattern. The tracheal mucociliary transport flow primarily moved along the posterior wall and both lateral walls in a zigzag trace. Upon reaching the subglottis, the resin particles stayed underneath the vocal cords, and a whirlpool phenomenon developed. The majority of the particles were shifted and directed onto the posterior glottic area. With a short delay, some resin particles crossed over the free edge of the vocal cords and turned posteriorly along the medial upper cordal margin. No mucociliary transport could be observed on the entire upper surface of the true vocal cords, which is covered by squamous epithelium. Occasionally, a few resin particles in the vicinity of the epiglottic root traveled along the aryepiglottic folds toward the posterior commissure. All streams of mucociliary transport finally joined together in the interarytenoid area. After leaving the glottis, the resin particles traveled to the hypopharynx and entered the esophagus through the motion of deglutition. The pattern of mucociliary clearance in the laryngotracheal region was not delayed by stenting.

KEY WORDS — glottis, guinea pig, laryngotrachea, mucociliary transport, stent.

INTRODUCTION

As air pollution increases, so do the number of cases of upper respiratory tract and lung disease. Foreign particles that our body breathes in, such as dust, smoke, and various contaminants, all accumulate in the airway secretions and might hinder the laryngotracheal mucociliary transport. Studying the flow pattern of mucociliary transport will help to explain why certain areas of the laryngotracheal region are susceptible to diseases. For example, laryngeal tuberculosis is known to be spread directly to the posterior larynx by infectious airway secretions. This could be explained by the fact that the majority of the airway secretions passes through the posterior

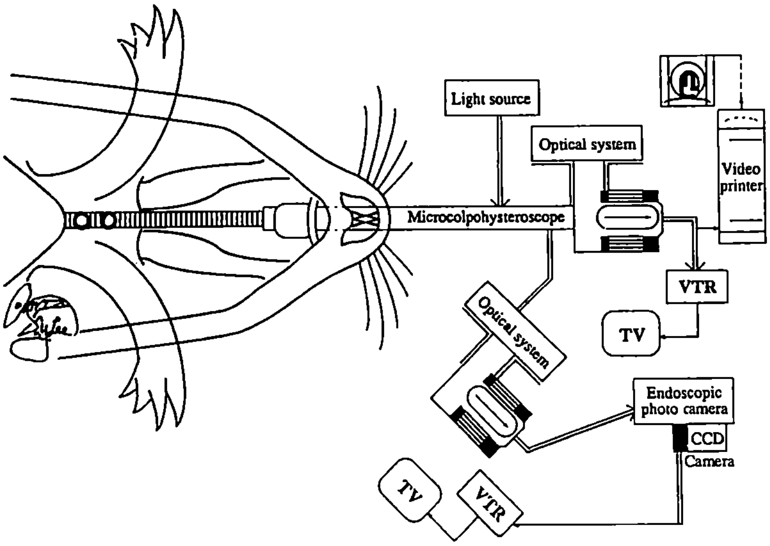

Fig 1. Block diagram of experimental animal model for observation of mucociliary transport pathway. VTR — videotape recorder.

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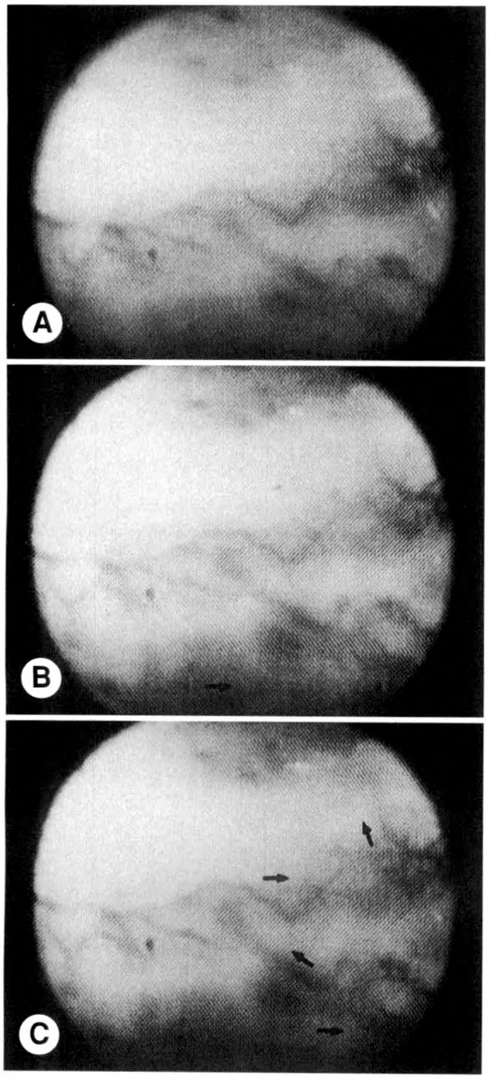


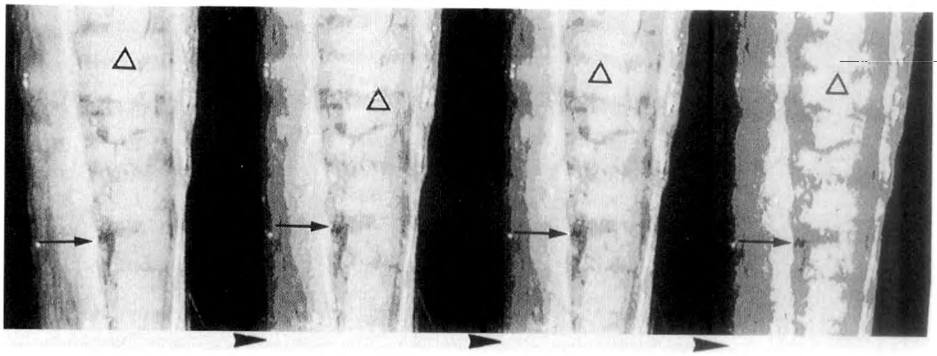
Fig 2. Serial pictures (every 30 seconds, from A-C) of cervical trachea. Mucociliary transport pathway was traced in zigzag-flowing pattern (arrows).

ommissure.[1] In addition, the foreign particles cap-ured by airway secretions are carried by mucociliary ransport, sent into the hypopharynx, and then swal-owed. If an infectious substance stagnates on the ucus blanket and is not removed immediately, it ill give chronic irritation locally. Thus, the pattern f transport of mucus in the laryngeal area might have a strong correlation to some laryngeal diseases.

With an endolaryngeal microscopic viewing and recording device, the present experimental model was designed to observe the laryngotracheal mucociliary transport in vivo and immediately postmortem by a nonradioactive marker technique. In a pilot experiment in the guinea pig, we successfully observed the sphincteric action and cordal movement of the glottis during respiration.[2] The same procedure was used to investigate the mucociliary clearance of the laryngotracheal tract, and we found that laryngotracheal stenting did not disturb the mucociliary clearance.[3] The purpose of this study was to elucidate the rheological characteristics of laryngotracheal secretions in vivo and with stenting.

MATERIALS AND METHODS

The experimental procedures were as described in a previous report.[3] In brief, guinea pigs weighing between 300 and 460 g (average 350 g) were used. After overnight fasting, the guinea pigs were injected intraperitoneally with pentobarbital sodium (50 mg/mL), 25 mg/kg of body weight. The depth of anesthesia was maintained at the level of spontaneous breathing without a coughing or vomiting reflex. The room temperature was maintained at 26°C. The guinea pigs were placed in the supine position. A small pillow was placed under the guinea pig's shoulder to help in extending the neck. This in turn helped straighten the animal's airway from the oral cavity to the larynx. The next step was to shave off the fur around the animal's neck and to sterilize the skin. Two tracheal openings were fashioned. The upper tracheotomy was for the placement of resin particles. The second tracheotomy was made about 0.5 cm below the first, in order to prevent cyclic interference from the respiratory airflow. The transport pattern of the resin particles was observed by inserting a Hamou's microendoscope (4 mm in diameter) through the guinea pig's oral cavity and into its larynx. The light source was a 250-W xenon light bulb and an optical fiber. The endoscope was connected to a charge-coupled device (CCD) and a video camera



ig 3. Tracheal specimen has been pened posteriorly. Photographs eated with "art" technique show creased contrast of resin particles. Flow pattern can be clearly een (arrows). Dark trace demonrates zigzag flow of particles lark color) vertically along lat-al wall. Transport stream was arly absent anteriorly (open tri-gles). Arrowheads indicate ronological order of photos.

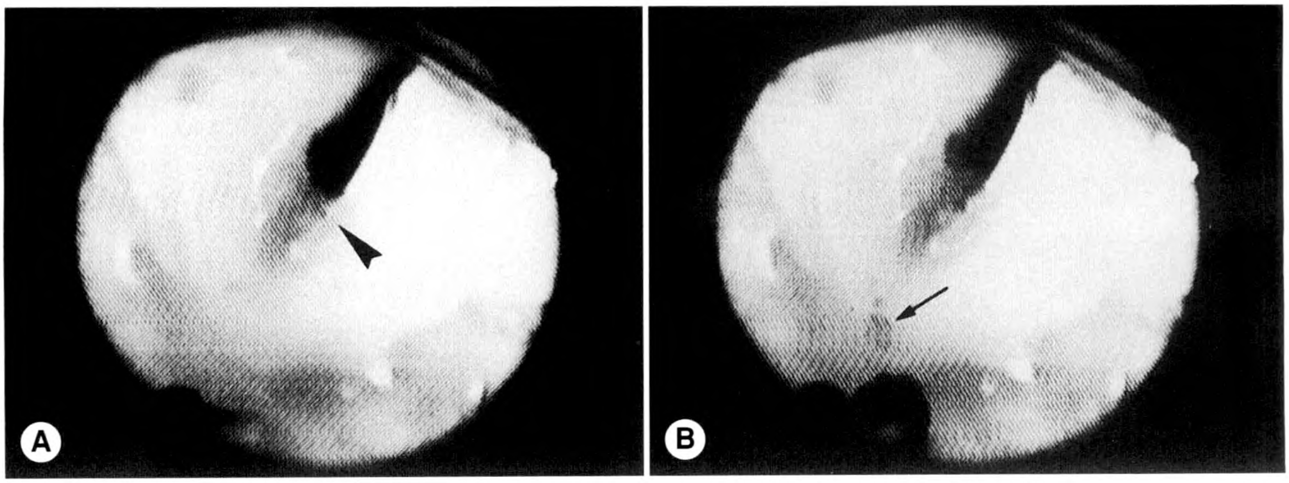


Fig 4. A) Immediate postmortem specimen demonstrates transport streams along cordal edge that join at posterior commissure. There is accumulation of resin particles at posterior commissure (arrowhead). B) Stream travels from posterior glottis to supraglottis and hypopharynx (arrow).

(Fig 1). The endoscopic laryngeal images were recorded by a video recorder (U-matic, 3/4 inch, Sony V.0.9600). Simultaneously, an automatic focusing photo camera (F810, Nikon) was used to produce a set of supplementary pictures as a reference. All recordings started from the moment the endoscope was inserted. The recorded images could be viewed via a monitor while the automatic photo camera was taking a picture every 30 seconds. The same procedures were performed on the animal with or without intralaryngeal stenting. The mucociliary clearing function at the levels of the trachea, subglottis, glottis, and supraglottis could be observed sequentially. The same recording techniques were also applied on several postmortem, opened laryngotracheal specimens.

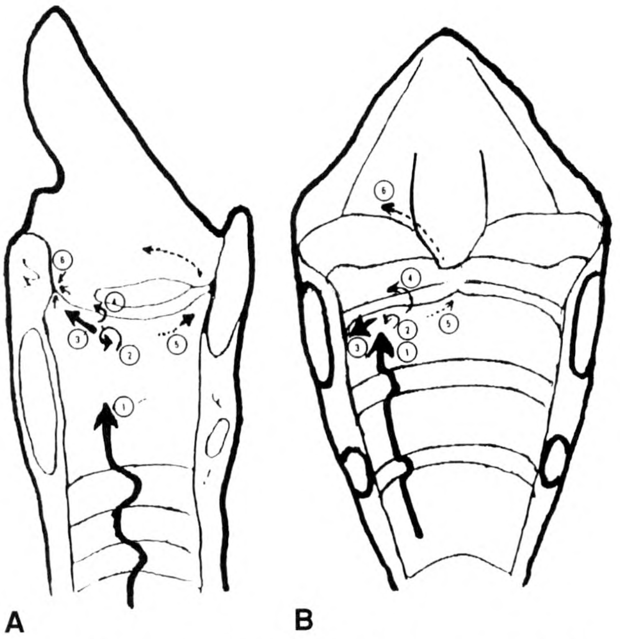


Fig 5. Summary of mucociliary flows in A) lateral and B) posteroanterior views of larynx. Tracheal mucociliary transport flow primarily moved along posterior wall and both lateral walls in zigzag trace (trace 1). Upon reaching subglottis, resin particles stayed underneath vocal cords, and whirlpool phenomenon developed (trace 2). Majority of particles were shifted and directed onto posterior glottic area (trace 3). Some resin particles crossed over free edge of cords and turned posteriorly along medial upper cordal margin (trace 4). Occasionally, some resin particles passed through anterior commissure (trace 5) and then, in vicinity of epiglottic root, traveled along aryepiglottic folds toward posterior commissure (trace 6). All streams of mucociliary transport finally joined together in interarytenoid area.

RESULTS

The dark secretion on the image was a mixture of mucus and resin, and accordingly made it possible to detect the mucociliary transport pattern in the airway. We found that few streams crossed the midline. Almost no resin was noted along the anterior wall

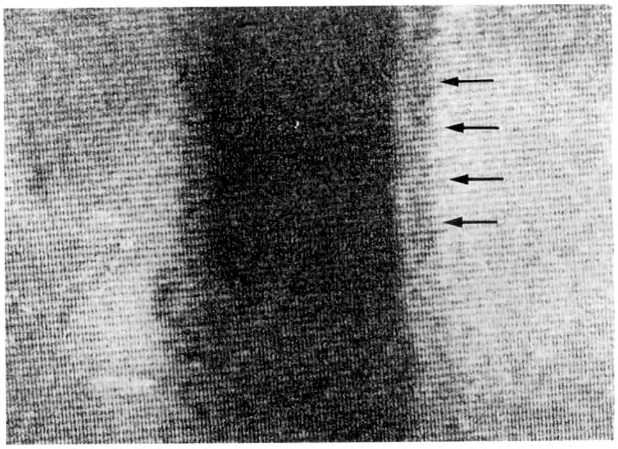


Fig 6. Mucociliary transport also passes along superior and inferior edges (arrows) of vocal cords, reaching posterior commissure and joining main posterior tracheal stream, where they mix together.

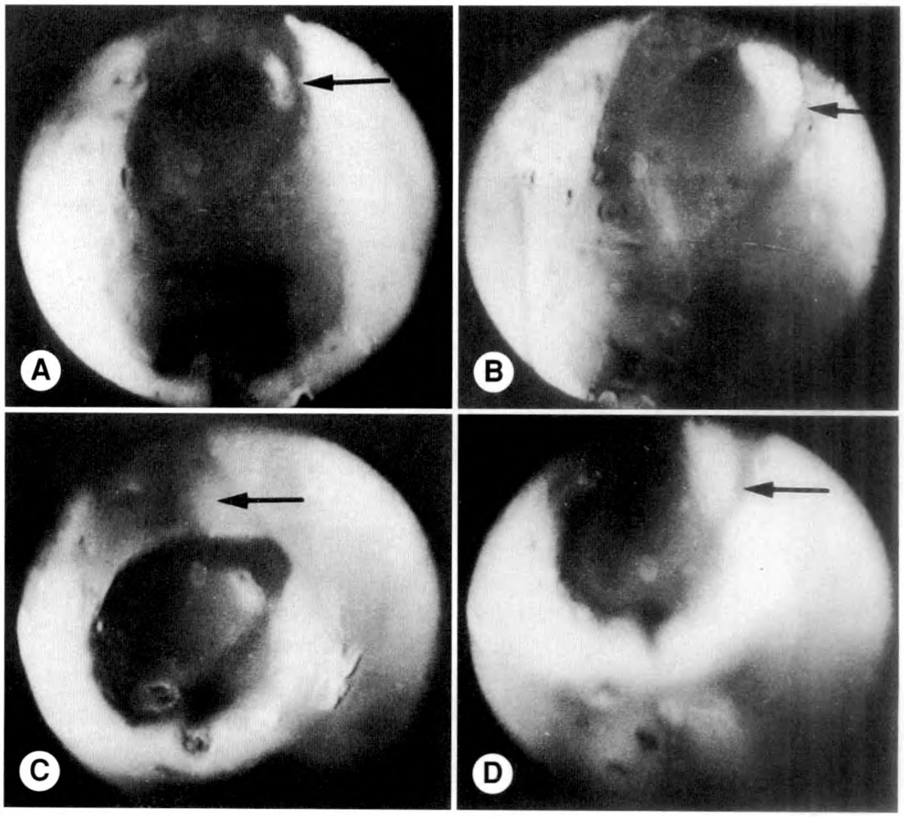


Fig 7. A) Intralaryngeal stent (arrows) was inserted in larynx with its upper end above level of vocal cords, o ventricular folds. B) Major transport stream on posterior wall traveled from subglottis to glottic edge, accumulating at interarytenoid region. C) During swallowing action, larynx ascended and stent was elevated upward. Top of stent moved secretions with resin particles from glottis hrough posterior commissure. D) Dynamic cleansing function of larynx is assisted by action of stent.

up to the level of the subglottis or anterior commissure. Most of the mucus flowed in a zigzag trace on he posterior and lateral walls vertically (Fig 2). For demonstration of the tracheal mucociliary transport, we treated the video image with an "art" technique o augment the dark color of the resin particles by ncreasing the contrast against the background (Fig 3). The video images were developed into photographs for observing and comparing the transport pattern of the particles and mucus on the glottis. Two photographs taken at intervals of 30 seconds are shown in Fig 4. The streams at both lower borders of the vocal cords turned sharply to the posterior direction, forming a "whirlpool" phenomenon, proceeded to the posterior commissure, and then joined the posterior subglottic flow at the interarytenoid region. The mucus streams crossed over the posterior glottis and traveled into the supraglottic area and then the hypopharynx (Fig 4).

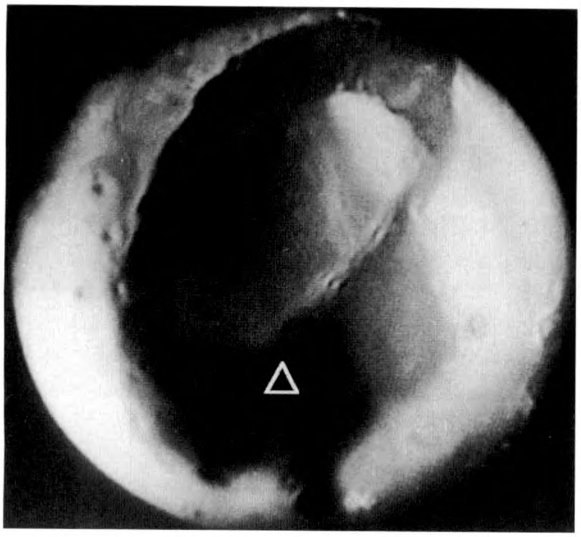


Fig 8. Mucociliary transport pathway of postmortem subject revealed same pattern as in vivo. Stenting did not disturb transport flow posteriorly between stent and luminal wall (triangle).

A simplified sketch of the transport process of the mucus is shown in Fig 5. It depicts upward movement of the lateral transporting streams. Most of the streams traveled from anterior to posterior and proceeded directly to the posterior commissure. Eventually, all the resin particles were moved away from the laryngeal area and accumulated in the hypopharynx and pyriform sinus. A small amount of resin particles moved toward the anterior commissure and continued to move toward the border of the epiglottis.

There was rotatory mucociliary transport action (whirlpool) in a few small focal areas of the upper trachea near the subglottis. The speed of transit appeared to be low because of dehydration or the increasing viscosity of mucus in the region of the vocal cords. From the close-up images taken in the lower cordal region, we observed that the resin particles gathered around the anterolateral part subglottically.

The mucus accumulating in the lower region thus formed a whirlpool effect. The flows also passed along the superior and inferior (Fig 6) edges of the vocal cords to reach the posterior commissure, then joined the main stream along the posterior tracheal wall. The secretions composed of resin particles, mucus, and air bubbles stayed at the interarytenoid region and clearly were retained there longer than on the tracheal wall. None of the resin particles were observed to cross over the vocal cords and reach the upper surface at this time. Only after a short delay could the resin particles cross over the entire free edge of the vocal cords and reach the upper surface.

In the stented group, the intralaryngeal stent was inserted in the larynx of the live subject with its upper end reaching above the vocal cord level to the ventricular folds. The major transport stream on the posterior wall traveled from the subglottis and both edges of the glottis, accumulating at the interarytenoid region (Fig 7A,B). During the swallowing action, the larynx ascended and the stent was elevated, with the top end of the stent helping to evacuate secretions with resin particles from the glottis through the posterior commissure (Fig 7C,D). The findings in postmortem subjects revealed a similar transport pathway as in vivo. The secretion passed through the interarytenoid region to the supraglottic region (Fig 8).

DISCUSSION

Although knowledge of the mucociliary transport in the trachea and larynx has been gathered through animal research, we still have limited information on the cleansing ability of the tracheal and laryngeal mucociliary function in humans. In 1966, Ewert and Martensson[4] observed the flow of mucus under the glottic area by placing a transconioscope through the cricothyroid membrane. Later, measuring the effectiveness of the mucociliary cleansing ability in humans was made easier and more accurate by introduction of a radioactive tracer and the use of gamma ray–emitting isotopes as labeling particles.[5] However, the use of radioactive isotopes is troublesome, and its potential for causing tissue damage is still a significant concern.

The mechanical process of coughing out particles from the trachea and the laryngeal area is complex. Bridger and Proctor[5] recall that Veale, in 1871, reported that voluntary efforts of coughing seem to contribute to expelling mucus only when the mucus is situated immediately beneath the epiglottis or just below or between the vocal cords. Actually, it is the mucociliary transport of mucus in the tracheal and laryngeal areas that renders the forceful cough effective in clearing out particles. Thus, the cleansing function of mucociliary transport is crucial in clearing particles from the trachea and larynx. Although swallowing does not clear particles from the larynx, drinking liquid would effectively wash the particles away from the entrance of the pharynx and larynx.

The laryngeal mucociliary cleansing procedure was not as simple or symmetric as that described above. Serial histologic section of the laryngeal area revealed that the squamous epithelium of the vocal cords did not extend to the anterior commissure. In contrast, the ciliated epithelium continuously extended upward from the subglottis via the posterior commissure to the supraglottis. The difference in the covering epithelia and distributing vessels might be one of the major determinants for the transport pattern. We found that the zigzag transporting trace of the trachea mainly came from the posterior part and proceeded perpendicularly upward. It was seldom seen in the anterior part. Therefore, a vertical tracheal incision is preferred when performing a tracheotomy. It might avoid interference with the mucous transport. The anterior commissure is not an area in which the transported mucus tends to linger. Lack of mucociliary function does not explain the frequent occurrence of cancer in this area. In particular, the majority of the mucus passed through the posterior commissure. This could explain why most laryngeal tuberculosis occurs in the posterior commissure. The glottis seems to be the major barrier to the transport of airway secretions. From this conclusion, we can infer that inserting an endotracheolaryngeal T-tube over the vocal cords will not slow the mucous transport. Instead, it tends to reduce the period of lingering in the subglottic region and speeds up the transport process.[3] According to the results of this experiment, an intraluminal stent with inward folding of the posterior wall will be useful in clinical practice from the point of view of laryngeal mucociliary transport. Many studies on the function of mucociliary transport were done on immediately postmortem subjects as in our experiments. These data were all gathered from an in vitro model. Unfortunately, we are unable to extrapolate the results to our experiment. Advances have been achieved in radioactive detectors and other types of gamma ray–emitting isotopes. Although it provides an easier and more accurate way to study mucociliary function under a relatively physiological state, we still have to be conservative about the harmful effects that radiation may cause.

In conclusion, the technique of using an endoscope to record rheological data in the airway from a living animal is satisfactory. Further study with this animal model will permit elucidation of the mucociliary function in the laryngotracheal region.

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EUROPEAN ACADEMY OF FACIAL PLASTIC SURGERY

The annual meeting of the European Academy of Facial Plastic Surgery will be held September 15-17, 2000, in Thessaloniki, Greece. For information, contact Prof Dr J. Daniilidis, ENT Dept, Aristotelian University of Thessaloniki-AHEPA Hospital, 540 06 Thessaloniki, Greece; telephone 0030/31/994.761-2; fax 0030/31/994.916.

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MYOSIN HEAVY CHAIN EXPRESSION IN HUMAN LARYNGEAL MUSCLE FIBERS

A BIOCHEMICAL STUDY

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Since the intrinsic laryngeal muscles in humans are involved in specialized functions, one may suppose that this would be associated with the expression of specific myosin heavy chain (MHC) isoforms, as has been reported for the rat, dog, and rabbit. In order to determine which MHCs are expressed in the human laryngeal muscles, biochemical analysis using sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed. Thyroarytenoid and posterior cricoarytenoid muscles were obtained from a 7-month-old infant and 4 adults. In the adult human laryngeal muscles, 3 bands were resolved identical to those previously described in the human limb muscles (I, IIA, and IIB MHCs). In contrast, muscles from the infant also expressed fetal MHC and a novel MHC not observed in other human skeletal muscles. This novel band migrated at the same level as the laryngeal MHC previously described in the rat. Since these 2 isoforms disappear in the adult, the persistence in the infant could be correlated with the immature development of laryngeal functions and, in particular, phonation.

KEY WORDS — development, human laryngeal muscles, myosin heavy chain, polyacrylamide gel electrophoresis.

INTRODUCTION

Myosin heavy chain (MHC) is the major component of the thick filament of the sarcomere. The MHC content of a muscle determines its contraction speed, which is correlated with the activity of myofibrillar actomyosin adenosine triphosphatase (ATPase) at different pH levels.[1-3] Type II fibers display high myofibrillar ATPase activity at alkaline pH and low activity at acid pH (fast-contracting) and are characterized by the expression of either fast IIA or fast IIB MHC, whereas type I fibers exhibit the inverse (slow-contracting) and contain slow MHC.[4] Adult human limb muscles contain predominantly fast fibers (60%). However, some human muscles, such as the masseter,[5] diaphragm,[6] and posterior cricoarytenoid (PCA) muscles of the larynx,[7-12] have a predominance of slow type I fibers.

Different MHC isoforms (embryonic and fetal/neonatal)[3,13-15] have been identified both during development and in diverse adult muscles, and these MHCs can be identified by improved electrophoretic techniques.[16-18] However, in mammals, certain adult skeletal muscles, such as the extraocular,[19,20] tensor tympani,[21] and jaw[22,23] muscles, have been found to express a panel of tissue-specific MHC isoforms. The expression of these unusual MHC isoforms is thought to be related to the specialized functions of these muscles.

The intrinsic laryngeal muscles are involved in airway protection, breathing, and phonation, all of which require a sustained activity and rapid contraction times. Several studies have indeed demonstrated the expression of a particular atypical pattern of MHCs. Electrophoretic studies in the dog have shown that single muscle fibers of the lateral cricoarytenoid muscles do not express 1 MHC, but coexpress several MHCs.[24] This has also been reported for the human masseter[22] and has been related to the specific and varied physiological functions involved in maintaining the jaw and involved with mastication and speech. Lucas et al[25] have also reported that in the rabbit laryngeal muscles there is an expression of the extraocular MHC. Moreover, using electrophoretic techniques, DelGaudio et al[26] have described the presence of a specific laryngeal MHC isoform, MHCIIL (MHC type II laryngeal), which is expressed in rat laryngeal muscles.

In order to determine which MHC isoforms are expressed in the human intrinsic laryngeal muscles, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Fetal MHC and specific laryngeal MHC were identified in the

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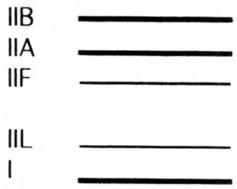

Fig 1. Schematic representation of electrophoretic mobility of different myosin heavy chain (MHC) isoforms. I, IIA, and IIB MHC isoforms are present in adult human laryngeal muscles. IIF (F, fetal) and IIL (L, laryngeal) MHC isoforms are, in addition, observed in muscles from 7-month-old infant. IIL MHC isoform is also present in rat laryngeal muscles.

laryngeal muscles from a 7-month-old infant, but these isoforms were never detected in the adult.

MATERIALS AND METHODS

Specimens. Intrinsic laryngeal muscles were obtained during autopsies of a 7-month-old infant and 4 adults (41, 54, 68, and 77 years old), in accordance with the French legislation on ethical rules. Thyroarytenoid (TA) and PCA muscles were excised from specimens within 30 hours of death. The TA, PCA, and soleus muscles were dissected out from a 2-month-old Sprague-Dawley rat (220 g). A previously well-characterized human masseter muscle from a 73-year-old adult was used to identify the position of migration of the different MHC isoforms. Muscles were frozen quickly by immersion in isopentane cooled with liquid nitrogen, and stored at −80°C.

Extraction Procedure. Minced muscle samples

[redacted]

blue) prior to migration.

5% SDS-PAGE. Five percent separating and 4.5% stacking gels were prepared from a solution of 30% acrylamide–0.6% bisacrylamide. Separating gels containing 35% glycerol were poured and were allowed to polymerize for 2 hours at room temperature.[16] Stacking gels were allowed to polymerize for 1 hour at room temperature and then 1 hour at 4°C. Muscle extracts (1 to 3 µL), from supernatants diluted into sample buffer, were boiled at 100°C for 1 minute prior to being loaded onto the gels. Electrophoresis was carried out for 24 hours at 120 V and at 4°C. Separating gels were silver-stained prior to being photographed.[27]

RESULTS

In the adult masseter muscle, 4 MHC isoforms can be identified: MHCIIB, MHCIIA, fetal MHC, and MHCI (Figs 1 and 2). In the adult human laryngeal muscles, 3 bands were resolved: the 2 slowest-migrating bands were identified as the fast MHCIIB and MHCIIA isoforms, and the fastest-migrating band as the slow MHCI isoform (Figs 1 and 2). MHCI and MHCIIA were predominant in the TA and PCA muscles, whereas MHCIIB was only a minor isoform. In the 7-month-old laryngeal muscle samples, 2 additional bands were observed, 1 of which corresponded to fetal MHC, and the other of which migrated between the fetal MHC and the slow MHCI isoforms (Figs 1 and 2). In order to identify this novel band, adult rat laryngeal extracts were made and com-

TA muscle than in the PCA muscle.

DISCUSSION

The intrinsic laryngeal muscles require specific neuromuscular substrates to perform highly sustained, coordinated, and rapid contractions. The specific contractile properties of muscles are correlated with their MHC content. The expression of the various MHC isoforms, which are both species- and tissue-specific, is regulated during development by a family of MHC genes.[28] The human laryngeal muscles have been previously characterized by several authors using histochemical approaches.[7-12] Following determination of their myosin ATPase activity,[4] it was found that the TA muscles contain the largest percentage of fast fibers, and the PCA muscles the lowest.[7-11] However, subdivision of the type II fibers in laryngeal muscles was found to be difficult with the classic myosin ATPase technique: Lacau St Guily and Fardeau[10] failed to distinguish subpopulations of MHCII (IIA, IIB) in human PCA and TA muscles. However, using a modification of this method called the inhibition reactivation myofibrillar ATPase technique, Claassen and Werner[12] were able to demonstrate a higher proportion of MHCIIA (fatigue-resistant) fibers in the TA muscles than in the PCA muscles. These authors also pointed out that there was a large degree of interindividual variability for these muscles.[10,12] Since, in the laryngeal muscles, it was difficult to classify the fibers into the 3 types of MHCs found in limb muscles (I, IIA, IIB), this could suggest the expression of additional MHC isoforms such as developmental MHCs or MHCs specific to the laryngeal muscles. With electrophoresis, it is now possible to identify all of the MHCs present in a muscle.[16-18]

In the rat, using such techniques, DelGaudio et al[26] were able to demonstrate that the rat laryngeal muscles express a specific MHC that they have called MHCIIL. We also found this atypical myosin isoform in rat laryngeal muscles. However, in the adult TA and PCA muscles in humans, only 3 bands could be resolved (Fig 2). This was similar to the pattern previously reported for the human biceps, corresponding to IIB, IIA, and I MHC isoforms.[13] A band corresponding to MHCIIL was never obtained in these adult samples. In contrast to the results observed in rabbit and canine laryngeal muscles, extraocular-type MHC was not observed in human laryngeal muscles.[24,25] In addition, it should be noted that even though the TA and PCA muscles differ in function, this difference is not reflected by their MHC composition.

Although no specific MHC isoform characterized the adult human laryngeal muscles, the current study demonstrates the expression of 2 additional MHC isoforms in the 7-month-old muscles.

Embryonic and neonatal MHCs are sequentially expressed during development and are replaced during fetal development by adult MHC isoforms. This sequential transition is controlled by the thyroid hormones.[29] However, persistence of "developmental" MHC isoforms is observed in certain normal adult muscles and in some muscle disorders.[30] In the human masseter, embryonic and fetal myosin isoforms are expressed at high levels during early infancy,[31] and persist in the adult muscle fibers.[21] In addition, embryonic and neonatal MHC isoforms are expressed in the adult extraocular muscles.[3,19] In this study, we also observed the persistence of a fetal MHC in the infant. This isoform is never observed in the adult laryngeal muscles, and its persistence at 7 months suggests that there is a delay in the regression of the fetal MHC. In limbs, this isoform is rapidly replaced by the adult fast and slow isoforms during the third trimester of fetal development and is usually absent soon after birth. Although, to our knowledge, no histochemical study using myofibrillar ATPase activity has been performed on young laryngeal muscles, previous investigations have shown an immature pattern of innervation in the human laryngeal myofibers, consisting of a poor arborization of nerve terminals until the third year of life and of a polyneuronal pattern of innervation.[32,33] The presence of a developmental MHC isoform in the infant laryngeal muscles, which is never observed in adults, could suggest a delayed maturation of the human laryngeal neuromuscular system and could be related to the progressive development of these muscles during the first few years of life.

A novel band was also observed in the human laryngeal muscles of the 7-month-old infant due to its migration at the same level as the MHCIIL found in rat muscles and was identified as human MHCIIL. This band was never observed in the adult muscles. Since this MHC is only transiently expressed, it could be hypothesized that the MHCIIL is also a developmental isoform that is gradually eliminated as the muscle becomes mature and acquires its adult functional properties.

Both in humans and rats, the percentage of MHCIIL was larger in TA muscles than in PCA muscles, and this finding could suggest a specific pattern related to the function of the TA muscle.

In the rat muscles, the diversity of myosin isoform expression during postnatal development has been reported in numerous muscles and has been correlated with both the origin and the activity pattern of the muscle.[34] In the diaphragm of the rat, the adult

MHC phenotype is not established until 115 days of life.[35] Consequently, the MHCIIL observed in laryngeal muscles of 2-month-old rats could be a developmental MHC that is tissue-specific, since this isoform was also exhibited in the laryngeal muscles of human infants. One could hypothesize that the MHCIIL may disappear in muscles of much older rats. Alternatively, this could reflect the fact that distinct contractile properties are needed during development and maturation that could vary greatly from one species to another. In particular, the development and the control of phonation is developed in humans, and not in rats.

This study demonstrates that the expression of specific MHC isoforms characterizes the development of the human laryngeal muscles, since fetal MHCs and an additional band, the MHCIIL, were only expressed in the infant. No specific MHC isoform was ever identified in the adult human muscles. The persistence of developmental MHC isoforms as in the human infant seems also to occur in rat laryngeal muscles. The disappearance of MHCIIL in humans could be related to the development of specific laryngeal properties in humans. Further investigations are needed to determine the exact sequence of MHC isoform transitions in human laryngeal muscles.

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HISTOPATHOLOGIC STUDY OF ALTERNATIVE SUBSTANCES FOR VOCAL FOLD MEDIALIZATION

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This research investigated the histopathologic and migratory properties of injectable alternatives for vocal fold medialization. Thirteen dogs underwent sectioning of the recurrent laryngeal nerve followed by vocal fold injection with 1 of 4 substances: Teflon, autologous fat, silicone suspension, or hydroxyapatite cement. Six months later, the animals were painlessly sacrificed and histopathologic analysis of the larynx and regional lymph nodes was performed. Although regional lymph node migration was noted, Teflon injection resulted in minimal vocal fold inflammatory reaction. Vocal folds injected with autologous fat exhibited persistence of fat at the injection site without significant inflammation or migration. Silicone suspension caused a localized giant cell reaction without regional lymph node migration, and 1 study subject died secondary to acute inflammation with critical respiratory compromise. Hydroxyapatite cement was well tolerated without inflammation or migration. This pilot study indicates that a wide range of possible substances for vocal fold medialization exist. Many of these may produce results superior to those obtained with Teflon and are thus far untested.

KEY WORDS — autologous fat, hydroxyapatite cement, silicone, vocal fold injection, vocal fold paralysis.

INTRODUCTION

The management of permanent unilateral vocal fold (VF) immobility remains controversial. Although otolaryngologists agree that medialization of the true VF into a functional phonatory and protective position is the treatment, no method to achieve this has become universally adopted. Currently, 3 major surgical options exist for the treatment of paralysis: VF injection, framework surgery, and reinnervation techniques.

The injection of space-occupying substances into the lateral VF is the oldest and most commonly used technique for VF medialization. In 1910, Wilhelm Brunings was the first to perform injection medialization with paraffin paste. Although he maintained that few complications resulted, others demonstrated the occurrence of paraffin granulomas with migration of the material to the regional lymph nodes and throughout the tissues of the neck. Because of these complications, the technique fell into disuse until reintroduced by Godfrey Arnold in 1962 with the injection of autologous cartilage paste in a similar fashion. However, the requirement for harvest and the tedious preparation of autologous material prompted the search for injectable synthetics.

Since its introduction in the 1960s, Teflon paste has been the mainstay injectable synthetic substance for VF medialization.[1] Several large patient series have demonstrated the clinical benefit of Teflon medialization with improvement in voice, cough, and ability for airway protection.[2-6] Although the majority of studies document improvement, evaluation of the clinical result has been compromised by the lack of uniform and objective measurement. In addition, negative effects from Teflon injection have been reported in 1% to 8% of patients.[7-9] These difficulties range from acute airway compromise in the immediate postoperative period to the late development of chronic inflammation and foreign body granuloma formation with declining clinical results. Further studies have shown stiffening of the VF with stroboscopically demonstrated disruption of the mucosal wave, migration of the Teflon to regional nodes, and a suboptimal long-term clinical result with further complications following interventions intended to treat granulomas.[10-14] Because these problems are attributable to the inherent properties of Teflon, alternative synthetic and autologous materials are currently being investigated.

Although a variety of materials, including colla-

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gen, autologous fat, and silicone, have been tested, a multitude of alternative materials exist. The ideal injectable substance would be biocompatible, nonreactive, nonvolatile, noncarcinogenic, and easily injected and would maintain its volume and position after injection. Based on these criteria, this research was designed to investigate 3 potential injectables, including hydroxyapatite cement (HAC), Macroplastique, and autologous fat.

Hydroxyapatite cement is a calcium-phosphate compound that crystallizes to a rigid consistency in an aqueous environment. It has been previously used in a wide variety of surgical settings, including orthopedic procedures, craniofacial reconstructions, ossicular replacement, and laryngotracheal reconstruction. Because this compound is found in normal bone, HAC has demonstrated biocompatibility, inducing only a mild inflammatory reaction without giant cell formation.[15] One histopathologic study demonstrated that 6 months after implantation, the surgical site had developed fibrosis equivalent to that occurring in any surgical wound.[16] In addition, the volume of HAC implanted has been shown to remain constant, with minimal fibrovascular proliferation replacement.[17,18] Migration of the substance has not been investigated. This protocol represents a new potential application for HAC.

Silicone has been tried for VF injection, although the substance is not currently approved by the US Food and Drug Administration for the clinical application of VF injection. Several international studies have inferred that it provides lasting clinical benefit, although other series report absorption of the liquid form.[19,20] Macroplastique (Uroplasty Inc, Minneapolis, Minn) is a solid polydimethylsiloxane textured particle that is larger in size than the silicone particles included in suspended gels. This product already has international patents for use in the treatment of gastric reflux, urinary incontinence, vesicoureteral reflux, and multiple plastic surgery applications. Although patented and approved in Europe for VF medialization, where unpublished results indicate efficacy and safety, Macroplastique is thus far not approved for this application in the United States. Histopathologic studies of the material have included cutaneous injection in rabbits, which demonstrated an initial inflammation persisting for 6 to 10 weeks, resulting in collagen replacement of the hydrogel carrier surrounding the individual particle masses.[21] Similarly, following human cutaneous injection, a low-grade inflammatory reaction is histologically noted that progresses to a quiescent giant cell reaction without asteroid body formation.[22] Specimens obtained from a limited number of patients 8 to 22 months after Macroplastique VF injection revealed no sign of inflammation, necrosis, fibrosis, or vascular changes in the surrounding tissue (W. Thumfart, unpublished findings). In addition, migration of Macroplastique has not been noted secondary to the relatively larger silicone particle size. This study formally evaluated migratory and local inflammatory properties of silicone suspension in VF injection.

Studies of autologous fat for VF medialization have demonstrated easy injection, uncomplicated retrieval in overinjection, and soft bulk in the VF producing a normal vibratory quality. A number of clinical series report the permanent improvement of speech, with a decrease in aspiration and glottic insufficiency, in patients following injection of autologous fat with a follow-up of several years.[23] Others report superior voice quality following the injection of fat as an alternative to Teflon because of relatively reduced stiffness of the VF.[24] Clinical symptoms did not recur with the partial reabsorption of injected fat, and with proper handling of harvested adipose tissue, the majority of injected fat persisted upon extended follow-up.[25] No study has documented the specific histopathologic response to autologous fat injection or migration of the substance.

This research was designed to evaluate the migration of and local tissue reactions to these alternative substances when used for VF medialization. Thirteen dogs underwent transection of the recurrent laryngeal nerve and injection medialization of the affected VF with 1 of the test substances. After 6 months, medialization permanence, histopathologic consequences, and migratory properties of the injected materials were determined.

METHODS AND MATERIALS

All procedures and animal care were performed according to standards prescribed in the Good Laboratory Practice (GLP) guidelines in accordance with the Animal Research Committee at The Cleveland Clinic Foundation. Thirteen medium-sized (25 to 30 kg) mongrel dogs were divided into 4 study groups. On the designated operative day, the study subjects underwent gradual induction of general anesthesia (Pentothal 20 to 30 mg/kg) with documentation of normal true VF mobility and laryngeal anatomy. After intubation and adequate general anesthesia (halothane 1% to 2%), the operative area was shaved, prepared, and draped in a sterile fashion. A left-sided neck exploration was performed via a paratracheal incision to identify the recurrent laryngeal nerve. After identification, a portable nerve stimulator was used for verification. A 2-cm segment of the nerve was excised, and both the proximal and distal ends were suture-ligated to prevent reinnervation. Following discontinuation of the anesthetic agent, direct laryngoscopy confirmed immobility of the left VF, and after reinduction, the surgical incision was closed.

The test materials were subsequently prepared for injection. For each injection, a fresh sterile 3-mL syringe and an 18-gauge laryngeal needle were utilized to prevent inadvertent cross-contamination by other materials. The needle and syringe were subsequently loaded into a Brunings injector that did not have direct contact with any of the materials. Both the Teflon paste and the Macroplastique (Macroplastique silicone suspension and injecting supplies provided courtesy of Uroplasty, Inc) were supplied in ready-to-use form, and were loaded into the syringe and injection equipment. The HAC was prepared by mixing 10 g of the powder with 3 mL of sterile water to form a paste (National Institute of Standards and Technology, Gaithersburg, Md). This was then loaded into the syringe. The autologous fat was harvested from the lower neck by simple excision at the time of neck exploration. Approximately 3 cm^3 of adipose tissue was obtained, and after gravity drainage of serum and blood, this was prepared for injection by careful mincing to a fine consistency and then loading into the syringe.

With direct visualization, the denervated VF was injected at a single location at its most lateral posterior third. The injected material was ideally placed between the vocal process of the arytenoid and the thyroid cartilage. Approximately 0.5 to 1.2 mL of the selected substance was injected to effect adequate medialization as determined by direct laryngoscopy. Subsequent video documentation of the medialization was completed.

Postoperatively, study subjects were maintained for 6 months. Animals were then sedated and endoscopic visualization was performed to document VF immobility and the efficacy of the medialization. Subsequently, the study subjects were painlessly sacrificed and a total laryngectomy and bilateral lymphadenectomies were performed. Tissue was preserved in Histochoice solution and submitted for pathological analysis. Photographs of the gross pathological appearance were completed, the tissue was embedded in paraffin, and histologic sections of the larynx (2-mm sections) and bilateral associated lymph nodes were prepared. The tissue sections were stained with hematoxylin and eosin and examined with a standard light microscope and a polarizing microscope. Pathological analysis was completed by a single-blinded pathologist (J.M.) with special attention to the texture of the VF, the amount and type of the local inflammatory response, and possible migration or lymph node reaction. Inflammatory reaction was described qualitatively in terms of mild, moderate, or severe on the basis of its intensity.

RESULTS

Preoperative direct laryngoscopy documented normal bilateral true VF mobility and laryngeal anatomy in all study subjects. Following nerve sectioning, all left VFs demonstrated immobility. At the end of the study period, all denervated true VFs remained immobile but medialized.

Twelve of the 13 study subjects survived until the designated sacrifice day, and all of these exhibited grossly normal vocalization. One animal died on postoperative day 2, following an uneventful procedure utilizing silicone suspension. Although this animal did well immediately postoperatively, over the ensuing 2 days, progressive stridor and respiratory difficulty occurred with eventual respiratory insufficiency and death. These symptoms occurred and progressed despite the administration of multiple steroid pulses (Decadron 12 to 20 mg). Postmortem gross examination of the larynx revealed an edematous tissue reaction in the supraglottic and subglottic regions of the left true VF causing airway obstruction. Subsequent histology demonstrated extensive acute inflammation and tissue necrosis involving the superficial epithelium and extending deep into the underlying muscle and connective tissue. All other animals survived the postoperative period without complication. Irritation of the surgical incision in the neck occurred in the majority of animals, but upon its recognition, subsequent manipulation of the site was prevented by the placement of a soft cervical collar to limit access.

Gross pathology revealed that the injected material persisted in the true VF in all study subjects. All larynges demonstrated normal gross anatomy except for that of a single study subject (Teflon group), which demonstrated a 1.5-cm fluid-filled cavity (seroma) in the left false VF of unknown cause and significance. Histologically, all denervated VFs atrophied. In contrast, contralateral VF tissue was unremarkable or demonstrated only mild chronic inflammatory changes, most likely secondary to frequent loud vocalizations that may be compared to human voice abuse. Similarly, contralateral lymph nodes were considered normal or demonstrated only mild chronic inflammation or small amounts of contaminant material. Histologic results from injected VFs and ipsilateral lymph nodes are listed in the Table. The histopathologic results of Teflon injection, including chronic inflammation and migration, were in accordance with previous research (Figs 1A and 2). The VFs treated with HAC demonstrated persistence of

HISTOLOGIC ANALYSIS OF INJECTED VOCAL FOLDS AND IPSILATERAL REGIONAL LYMPH NODES

Material Injected	*Vocal Fold Histology*	*Regional Lymph Node Histology*
Teflon	Polarizable foreign material (Teflon) (3) Minimal chronic inflammation (1) No inflammation (2)	Polarizable foreign material with giant cell reaction (1) Unremarkable (2)
Hydroxyapatite	Crystalline foreign material (3) with osseous metaplasia (1) Moderate chronic inflammation (1) Minimal chronic inflammation (2)	Unremarkable (3)
Macroplastique	Nonpolarizable foreign material (3) Prominent giant cell reaction (3)	Unremarkable (3)
Autologous fat	Fat present (3) Minimal chronic inflammation (3)	Unremarkable (3)

Each group included 3 study subjects. Numbers in parentheses indicate number of animals demonstrating specified finding. Unremarkable lymph nodes did not harbor foreign material or inflammatory reaction.

the injectable in the VF without migration and with a limited chronic inflammatory response, similar to injection at other sites (Fig 1B). In contrast, although the silicone suspension did not exhibit migration to regional lymph nodes, injected VFs demonstrated prominent foreign body giant cell reactions (Fig 1C). Autologous fat remained in the VF with mild chronic inflammation (Fig 1D).

DISCUSSION

Use of the first VF injection material, paraffin, was plagued by subsequent development of chronic inflammation, granulomas, and regional migration. In many respects, 75 years later, the currently popular

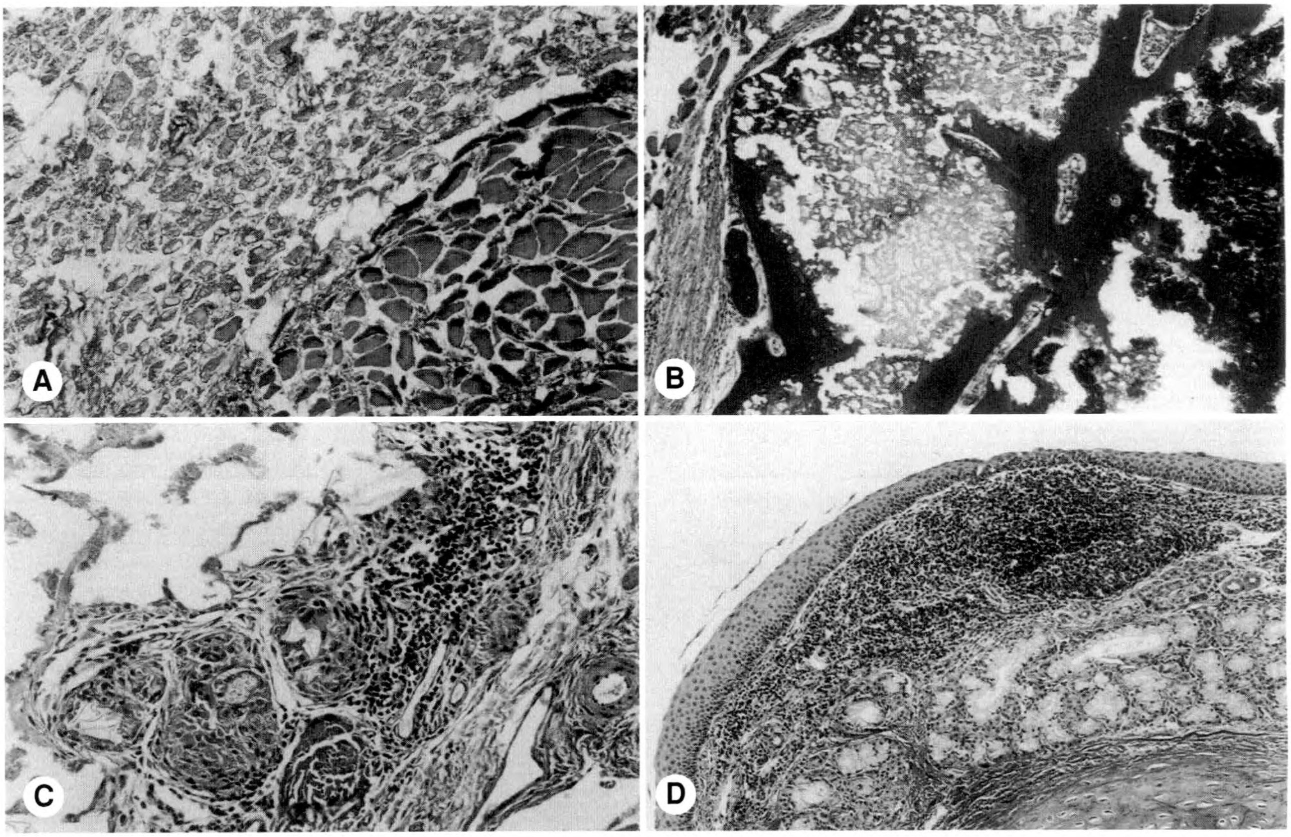

Fig 1. Histologic analysis of injected vocal folds. All specimens are paraffin-fixed and hematoxylin-eosin–stained. A) Vocal folds injected with Teflon demonstrated only mild inflammatory reaction, equivalent to histology of noninjected vocal folds (original ×100). B) Vocal fold tissue surrounding hydroxyapatite cement developed osseous metaplasia without significant inflammatory reaction (original ×100). C) Silicone suspension induced giant cell inflammatory reaction surrounding foreign material (original ×200). D) Autologous fat–injected vocal folds revealed baseline chronic inflammation with persistence of injected adipose tissue (original ×100).

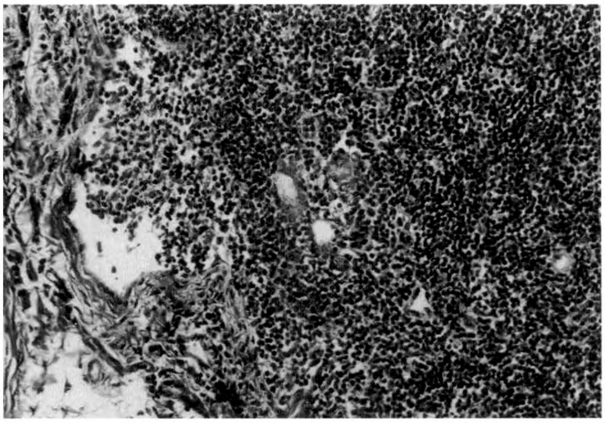

Fig 2. Consistent with findings of other histologic studies, Teflon was noted to migrate to regional lymph nodes within 6 weeks of injection (original ×200).

synthetic materials offer few improvements. Although the mechanical properties of VF medialization by injection produce favorable results, the search continues for a material that will permanently provide this bulk effect and space-occupying function for VF medialization without associated complications. Therefore, this research was designed to preliminarily evaluate the potential qualities of and tissue reaction to several alternatives to Teflon.

Control study subjects injected with Teflon demonstrated results comparable to those of previous research.[2,11] A local inflammatory response (mild to moderate chronic inflammation) was noted to be somewhat attenuated when compared to previous histologic studies, but this may have been secondary to the abbreviated study period. Regional lymph nodes demonstrated migration of the Teflon to adjacent lymph nodes, as has been previously shown. These results not only assured us of dissection and excision of correct lymph node masses in the lateral neck, but also provided the standard to which other materials could be compared.

We chose HAC for inclusion in our selected study materials secondary to its nonreactivity and firm consistency. Usage of the material required immediate preinjection mixing of the material with sterile saline, but ready dissolution and uniform injectability were demonstrated. In accordance with previous studies, local reaction was negligible, and in this research migration was not detected. Although some may argue that the excessively firm consistency of the material may prove detrimental to the mucosal wave, if it is injected in the posterior lateral location as described above, this firmness should not be of clinical concern. More importantly, the absence of an acute or chronic inflammatory response to HAC may preserve VF texture and therefore the normal mucosal wave.

The characteristics and tissue reaction observed following silicone suspension injection did not indicate that this was a significant improvement over Teflon, despite excellent clinical results reported abroad. The expiration of a study subject on postoperative day 2 was related to an acute inflammation causing airway compromise that was not responsive to steroids or other conservative measures. This response, if realized to a similar degree in a clinical subject, would have clearly required intubation or tracheostomy to prevent mortality. Although isolated similar cases of acute airway compromise requiring surgical intervention following injections of Teflon and other substances have been reported, the incidence is exceedingly rare. Moreover, final histologic analysis revealed a more intense inflammatory reaction than that resulting from Teflon injection, with significant giant cell reaction and the early signs of granuloma formation. The results of our research suggest that the use of silicone suspension presents similar difficulties and disadvantages as those noted with Teflon, and therefore may not constitute a significant improvement over the latter.

Finally, the use of autologous fat demonstrated favorable results. Upon injection, a pliable fullness of the VF was obtained that provided favorable medialization. Although some studies have demonstrated a loss of fat mass over time, our study showed persistence of the autologous fat at the injection site. Moreover, pathological analysis did not detect migration, and little appreciable local inflammatory response was noted.

The results of this preliminary study could have been made more definitive by the application of more study materials, inclusion of a larger number of study subjects, and extension of the study period. However, the protocol was designed to provide preliminary results that might lead to more extensive work by our group and others. Moreover, although qualitative or objective data concerning the voice might have added to the final analysis, the goal of this study was histopathologic in nature. Clearly, further research must be undertaken before definitive statements are made. However, although no conclusion concerning injectables can be made on the basis of this study, preliminary findings indicate that HAC and autologous fat produced a minimal degree of inflammation without detectable migration. These materials may, therefore, be worthy of further investigation. In addition, the results of this study and a review of the literature indicate the existence of a wide range of autologous and synthetic materials that may provide clinical benefit in VF injection while minimizing adverse histopathologic consequences.

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SINUSITIS AND CAROTID ARTERY STROKE

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The relationship between sinusitis and ischemic stroke is unexplored. The anatomic proximity between the paranasal sinuses and the internal carotid artery suggests that inflammation of the sinuses could easily extend to the intracranial vasculature. We report 4 patients with acute ischemic stroke and extensive disease of the paranasal sinuses. All patients had large vessel stroke involving the internal carotid artery territory. All patients had extensive disease of the sphenoid and other sinuses. The sinus disease was demonstrated by magnetic resonance imaging. These case report observations suggest a relationship between inflammation of the paranasal sinuses, particularly sphenoid sinusitis, and ischemic stroke.

KEY WORDS — carotid artery, inflammation, sinusitis, stroke.

Acute ischemic stroke is associated with extensive fungal infection of the paranasal sinuses.[1-3] The relationship between less severe sinus disease and stroke is unexplored. Recent work has described a relationship between respiratory infection, dental disease, and ischemic stroke.[4-7] We report on 4 patients, encountered during 1995 and 1998, who had sinusitis, stenosis or occlusion of the ipsilateral internal carotid artery, and acute ipsilateral brain infarct.

CASE REPORTS

Patient 1. A 45-year-old woman was admitted to the Los Angeles County–University of Southern California Medical Center (LAC-USC) for sudden-onset slurred speech and left-sided weakness and headache. She had no history of hypertension, diabetes, smoking, heart disease, or migraine; she had a prior stroke with complete resolution. She reported having episodes of sneezing, nasal stuffiness, and increased nasal secretions for the previous several years. Neurologic examination showed sensorimotor deficit with left-sided hyperreflexia. Magnetic resonance imaging (MRI) demonstrated the new infarct, as well as acute inflammatory sinus disease, with an air-fluid level in the sphenoid sinus and mucosal thickening of the right maxillary sinus (Fig 1). A magnetic resonance angiogram (MRA) showed occlusion of the right internal carotid artery. She had an uneventful hospital course. Ten months later, she had a residual mild left hemiparesis.

Patient 2. An 11-year-old boy with no significant past medical history was admitted to Children's Hospital of Los Angeles with weakness of sudden onset. Neurologic examination revealed expressive aphasia and a mild right-sided motor deficit. Magnetic resonance imaging showed a left middle cerebral artery territory infarct. There was an air-fluid level of the left maxillary sinus with severe mucosal thickening of the ethmoid and sphenoid sinuses (Fig 2). He remained stable during hospitalization with resolution of aphasia. He was discharged on aspirin. The patient required additional hospitalization for recurrent stroke symptoms within the following 2 weeks. Labo-

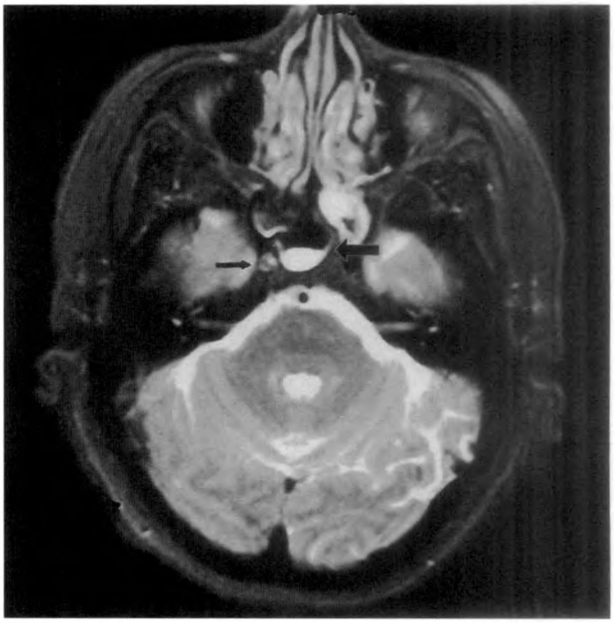

Fig 1. (Patient 1) MRI demonstrates air-fluid level in sphenoid sinus (thick arrow). Note lack of signal in right carotid artery (thin arrow), suggesting occlusion or tight stenosis.

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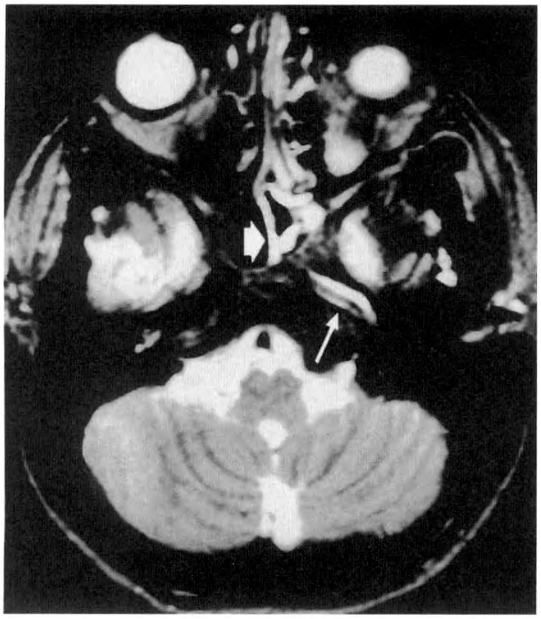

Fig 2. (Patient 2) MRI demonstrates ethmoid and sphenoid sinus disease (thick arrow) and impaired flow of internal carotid artery (thin arrow).

ratory studies indicated the presence of a lupus anticoagulant.[8] Conventional angiography showed severe stenosis of the left internal carotid artery. His hemiparesis and mental status improved and he was discharged 4 weeks after admission.

Patient 3. A 58-year-old woman was admitted to LAC-USC Medical Center following 2 episodes of transient weakness and numbness on the left side of her body with slurred speech. She had a history of headache, prior stroke, angina, hypertension, diabetes, and hypercholesterolemia. Neurologic examination on admission revealed slurred speech and mild left-sided motor deficit, which resolved within 2 hours. Twelve hours after admission, she developed severe left-sided weakness, sensory deficit, and dysarthria. Magnetic resonance imaging showed acute infarction of the right globus pallidus and internal capsule, and also complete opacification of the right sphenoid sinus (Fig 3). An MRA showed occlusion of the right internal carotid artery. A carotid duplex study revealed occlusion of the right internal carotid artery and stenosis of the left internal carotid artery. At the time of discharge, she had left hemiparesis.

Patient 4. A 62-year-old woman was admitted to LAC-USC Medical Center with generalized weakness, anemia, and a respiratory tract infection. She had a history of hypertension, diabetes mellitus, hyperlipidemia, and endometrial cancer. Five days after admission, she developed sudden-onset right hemiparesis and aphasia. An MRI scan demonstrated recent infarction of the left middle cerebral artery territory. It also showed extensive mucosal thickening of the left sphenoid sinus and severe narrowing of the ipsilateral internal carotid artery (Fig 4). She remained stable during her hospitalization and was discharged 24 days later.

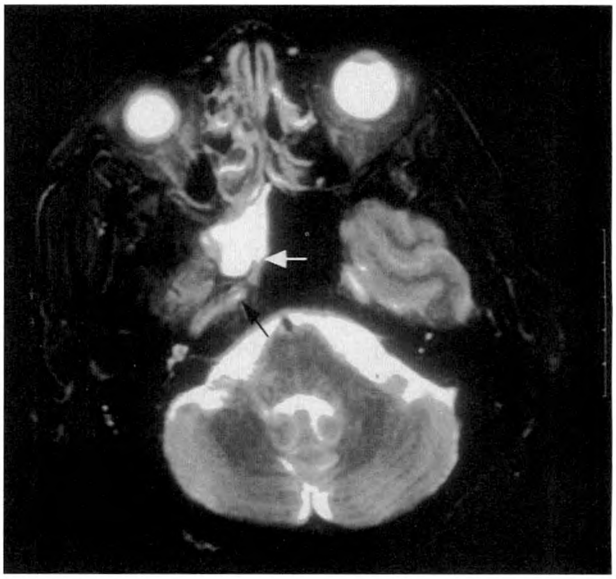

Fig 3. (Patient 3) MRI demonstrates sphenoid disease (white arrow) and impaired flow of internal carotid artery (black arrow).

DISCUSSION

Our 4 patients all had MRI evidence of severe disease of the paranasal sinuses, with few symptoms of sinusitis.[9] The sphenoid sinus was involved in all patients. All had acute ischemic stroke secondary to tight stenosis or occlusion of the internal carotid artery adjacent to involved paranasal sinuses. Taken together, these cases suggest an association between paranasal sinus disease, particularly sphenoid sinusitis, and ischemic stroke due to carotid artery disease.

The paranasal sinuses are, with the exception of the maxillary sinuses, separated from the cranial cavity by a thin bony wall.[10] The internal carotid artery lies immediately adjacent to the lateral wall of the sphenoid sinus[11] (Fig 5). True prolapse of the internal carotid into the sphenoid sinus, with the 2 structures separated by thin bone, is seen in 20% of cases.[12] Bony dehiscence may also occur, producing direct contact of the artery with sinus mucosa.[13,14]

There are also important relationships among the internal carotid artery and other paranasal sinuses. The internal carotid artery may bulge into the posterior ethmoid cells, separated by bone as thin as 0.1 mm; bony dehiscence occurs in some cases.[11,12,14] Anterior ethmoidal cells extend along the medial roof of the maxillary sinus, adjacent to the natural ostium of the maxillary sinus.[10] Maxillary sinusitis is typically due to infection within the anterior ethmoid

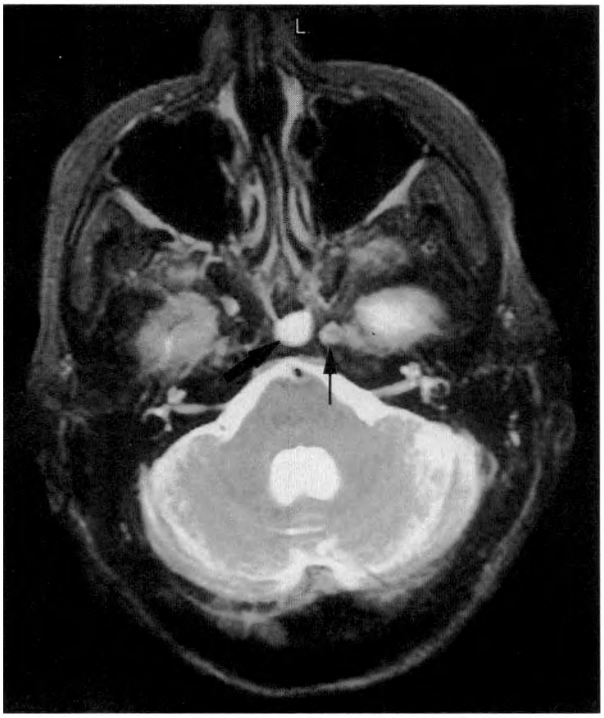

Fig 4. (Patient 4) MRI demonstrates sphenoid sinus disease (thick arrow) and severe narrowing of left internal carotid artery (thin arrow).

cells.[15] Thus, there is substantial anatomic and functional proximity among the internal carotid artery and

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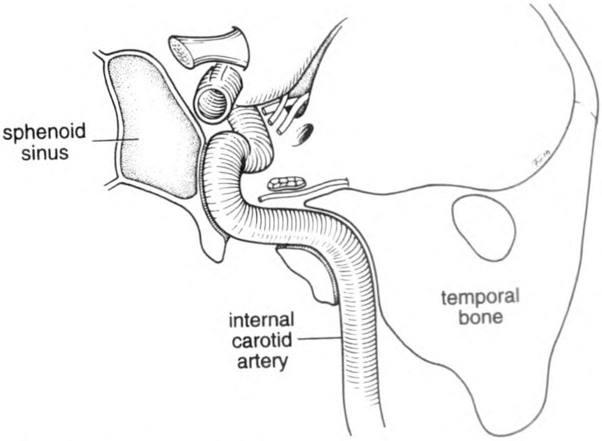

Fig 5. Anatomy of sphenoid sinus and internal carotid artery. Coronal view demonstrating proximity of internal carotid artery to sphenoid sinus.

of thrombosis at sites proximal or distal to the sinusitis.

Sinusitis may also serve as a marker of other infections (ie, respiratory tract and dental), the prevalence of which is increased in patients with acute ischemic stroke.[4-7] Sinusitis, shown by computed tomography or MRI, is more common in patients with respiratory tract illnesses.[17,18] Respiratory tract infections appear to transiently increase stroke risk by mechanisms that have yet to be fully elucidated.[19]

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IMAGING CASE STUDY OF THE MONTH

ANEURYSM OF AN ABERRANT RIGHT SUBCLAVIAN ARTERY PRESENTING AS DYSPHAGIA LUSORIA

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INTRODUCTION

"Dysphagia lusoria" is used to describe symptomatic extrinsic compression of the esophagus from any vascular anomaly of the aortic arch. It was first described by Bayford in 1787.[1] The term is derived from the Latin phrase *lusus naturae*, which translates as "trick of nature." The inherent suggestion in the use of the term is that of a potential error in ascribing dysphagia to this radiographic finding in the presence of another disorder. Although an abnormally sited vessel is present, it is thought that only 10% of these may cause symptoms due to compression.[2] It is important to remember that other common conditions such as achalasia, reflux, and esophagitis may need to be excluded as the cause of dysphagia. An aberrant right subclavian artery (ARSA) arising from the descending aorta is the most common cause of dysphagia lusoria, with an incidence of approximately 1% of the population. The anomaly is associated with an abnormal course of the right recurrent laryngeal nerve, which, instead of going under the right subclavian artery, travels directly to the larynx. This may put the nerve at risk during surgical exploration of the neck, especially during thyroidectomy.

Aneurysms of ARSAs are uncommon clinical entities, presenting infrequently to the otolaryngologist. Arterial aneurysms may develop wherever there is a marked weakening of the arterial wall. They may be due to congenital defects, trauma, systemic disease, or local infection such as atherosclerosis or syphilis. True atherosclerotic aneurysms of the subclavian artery are unusual. Occasionally, ARSAs have been described secondary to thoracic outlet compression.[3] Aneurysms may continue to increase in size until rupture, thrombosis, or distal embolization occurs.

The aorta and great vessels develop from the aortic sac, the ventral and dorsal aortic roots, and the arch arteries in the embryo. Congenital anomalies of the aortic arch have an incidence estimated in different sources to be between 3% and 3.5% in the general population and are usually asymptomatic.[4] The major branches of the arch of the aorta may number between 1 and 4, with various arrangements. In a series of 1,000 specimens reported in *Gray's Anatomy* by Anson in 1963, only 65% were found to have the usual arrangement. The commonest abnormal arrangement was only 2 branches, with the left common carotid artery arising from the innominate artery (27%). The next-commonest arrangement was 4 separate major branches (2.5%), in which the right subclavian artery arises from the left end of the arch and passes behind the esophagus. Rarely, all 4 branches may arise from 1 trunk, or there may be symmetric right and left innominate arteries.

The clinical features of an ARSA arise from its taking a retropharyngeal course to the right arm. These include intermittent dysphagia and gradual weight loss over a long period. Otolaryngologists in clinical practice are more familiar with the symptomatology of aberrant innominate arteries. Children may present with feeding problems, stridor, cyanosis, apnea, and recurrent pneumonia (in infants). This group of symptoms is known as "dying spells,"[5] and is due to tracheal compression by the aberrant innominate artery. Gross and Neuhauser[6] first described this cluster of symptoms in 1948, in children, in association with aberrant origin of the artery at a more distal site than normal on the ascending aorta. The other recognized means by which innominate aneurysms may occasionally come to the attention of the otolaryngologist is their invasion into the trachea,

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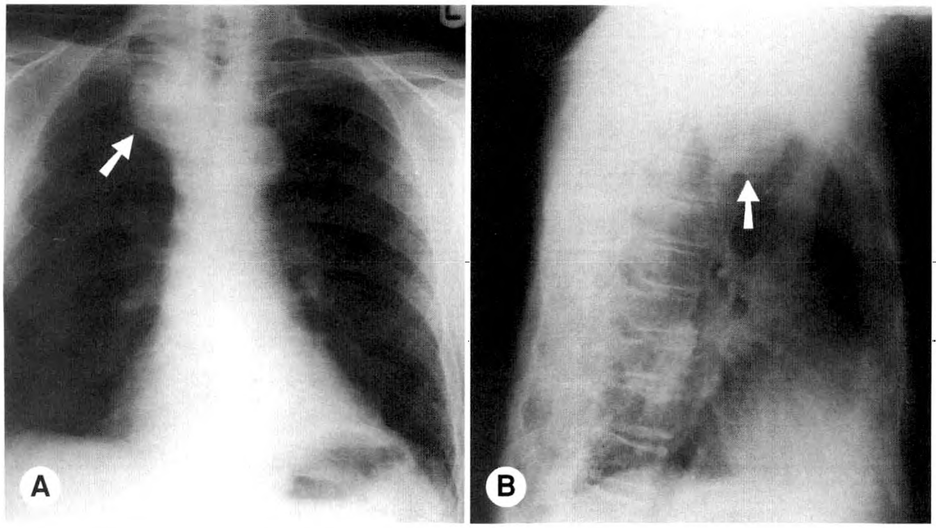

Fig 1. (Case 1) Chest radiographs. A) Anteroposterior, showing widened superior mediastinal contour (arrow). B) Lateral, showing apparent mass in superior mediastinum (arrow).

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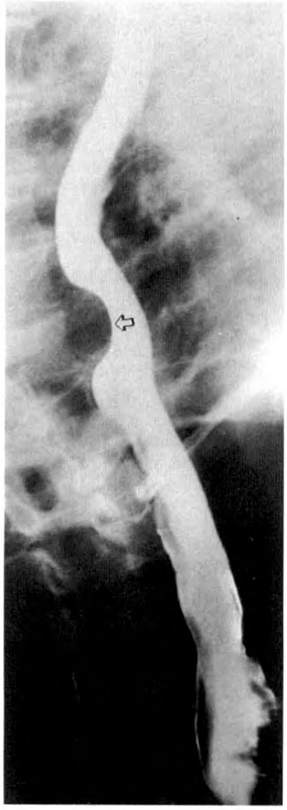

Fig 3. (Case 2) Oblique view of barium swallow study revealing classic appearance of posterior indentation of esophagus (arrow) by aberrant right subclavian artery.

ure 3 reveals the presence of an ARSA arising from the dorsal aorta, causing posterior compression of the esophagus. In contrast, anomalous innominate arteries cross the trachea anteriorly, causing anterior compression and respiratory symptoms. The left common carotid artery may arise from the aorta on the right or from the innominate artery and must also be considered in the differential diagnosis of vascular lesions causing anterior compression of the trachea. In a variant of this anomaly, the innominate and the right common carotid arteries arise from the same trunk, and following their division, both the trachea and esophagus are encircled, leading to respiratory obstruction and dysphagia.[9]

While the above techniques may at times simply suggest an abnormality, CT, magnetic resonance imaging, ultrasound, or angiography can be diagnostic. Magnetic resonance imaging is a useful noninvasive technique, capable of demonstrating flowing blood. Computed tomography may not be able to differentiate the vascular nature of a lesion such as this unless contrast is used, as the attenuation may be homogeneous.[10] Magnetic resonance imaging has the further advantage that radiation exposure is avoided. Because of its availability, however, and because it may be interpreted by general radiologists, CT remains the most common procedure utilized in the assessment of the abnormal mediastinal contour (Fig 2).

Ultrasound is a better modality in the delineation of aneurysms at other anatomic sites, especially peripherally and in the abdomen. Transesophageal ultrasound has a valuable role in the diagnosis of thoracic aortic dissection. A fixed neck curvature or the presence of a short neck may, however, diminish the quality of images produced with ultrasound.

SURGICAL MANAGEMENT

Surgical management requires a thorough understanding of the patient's anatomy and careful preoperative planning. Endoscopy is not required to establish a diagnosis, but it may be needed in individual cases to exclude other abnormalities. Classically, a pulsatile compression of the lumen is seen at esophagoscopy. If respiratory symptoms are present, bronchoscopy may be indicated. Methods described in the management of ARSA range from simple exploration of the vessel, to graft replacement with either prosthetic or autogenous graft materials, to ligation

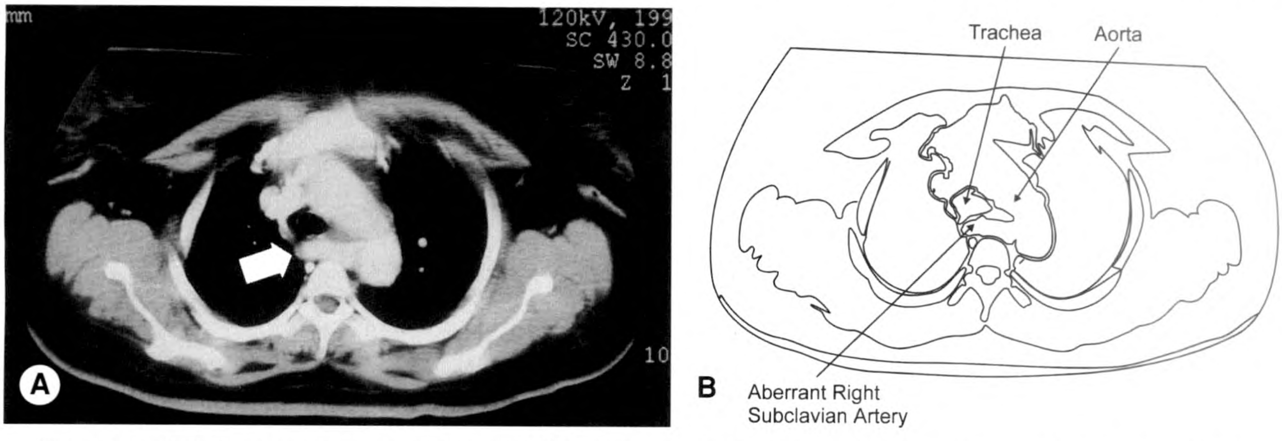

Fig 4. (Case 2) A) Axial contrast-enhanced computed tomography scan through upper thorax, showing aberrant right subclavian artery (arrow). B) Line drawing of A.

of the ARSA and anastomosis of the distal portion end-to-side to the right common carotid artery. In patients with thoracic outlet compression, excision of the first rib may be neccessary. One paper recommended the excision of the ARSA at its root through a right supraclavicular incision and reimplantation onto the right carotid artery without opening the chest.[11] Complications include hemorrhage, rupture of the aneurysm, and vocal cord paralysis. Late complications include stenosis of the graft. Bower et al[3] recommend elective isolated repair, and they state that this can be accomplished with a relatively low risk of mortality or morbidity. Five- and 10-year survival figures of 96.9% and 71.6%, respectively, were achieved in their series, for all subclavian artery aneurysm repairs, excluding operative deaths. The life expectancy of patients with dysphagia lusoria necessitates consideration of the long-term consequences of surgical intervention.[12]

CONCLUSION

Our case report exemplifies the value of imaging in the investigation of dysphagia lusoria. We describe 2 cases of ARSA, 1 of which was aneurysmal, in patients who presented to the otolaryngology clinic with dysphagia. We have found contrast-enhanced CT to be an accurate and sensitive modality in the investigation of extrinsic esophageal compression.

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CLINICOPATHOLOGICAL CONSULTATION

HYALINIZING TRABECULAR ADENOMA OF THE THYROID DIAGNOSED BY FINE-NEEDLE ASPIRATION BIOPSY

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Two cases of hyalinizing trabecular adenoma of the thyroid are presented. The diagnosis was established on initial assessment by fine-needle aspiration biopsy. Histologic evaluation of the surgical specimens confirmed the diagnosis of hyalinizing trabecular adenoma.

INTRODUCTION

After its description in the literature,[1,2] the term hyalinizing trabecular adenoma (HTA) of the thyroid was included in the World Health Organization's classification[3] and accepted by the Armed Forces Institute of Pathology[4] as an uncommon distinct benign epithelial lesion exhibiting a prominent trabecular arrangement and equally prominent stromal hyalinization. However, the recently demonstrated relationship between HTA and papillary carcinoma[5] and the reported cases of hyalinizing trabecular carcinomas[6-9] have added to controversy concerning the potentially malignant behavior of the tumor.

CASE REPORTS

Case 1. A 34-year-old woman had a palpable nodule in the left lobe of the thyroid detected on routine physical examination. A fine-needle aspiration biopsy

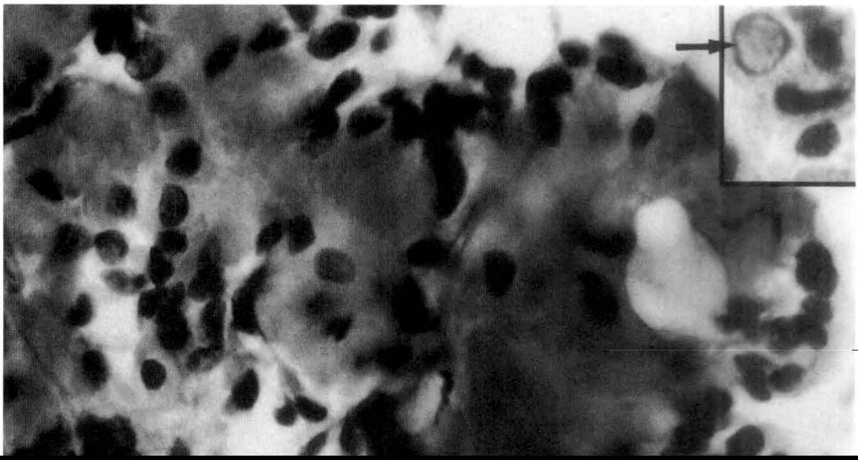

Fig 1. (Case 1) Fine-needle aspiration biopsy of nodule in left thyroid lobe (Papanicolaou, original ×240). Shows fragments of hyaline stroma resembling amyloid, intermingled with single cells or small groups of cells with uniform round-to-oval nuclei. Inset) Pseudoinclusion is evident in nucleus of neoplas-

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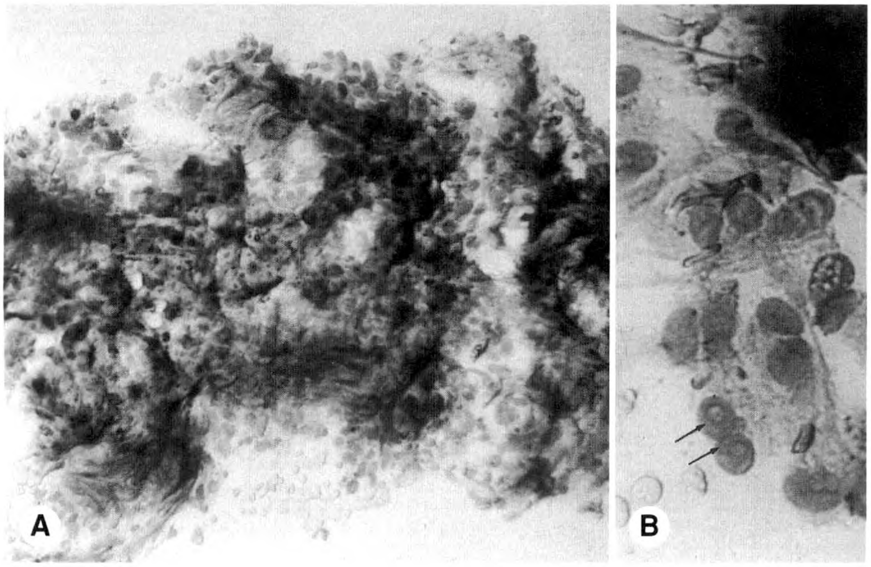


Fig 2. (Case 2) Fine-needle aspiration biopsy of nodule in right thyroid lobe. A) At low magnification, collected material appears very rich and is represented by numerous ovoid cells against background of fibrillated or amorphous hyaline material (May-Grünwald-Giemsa, original ×100). B) At higher magnification, some epithelial cells reveal pseudoinclusions (arrows; May-Grünwald-Giemsa, original ×400).

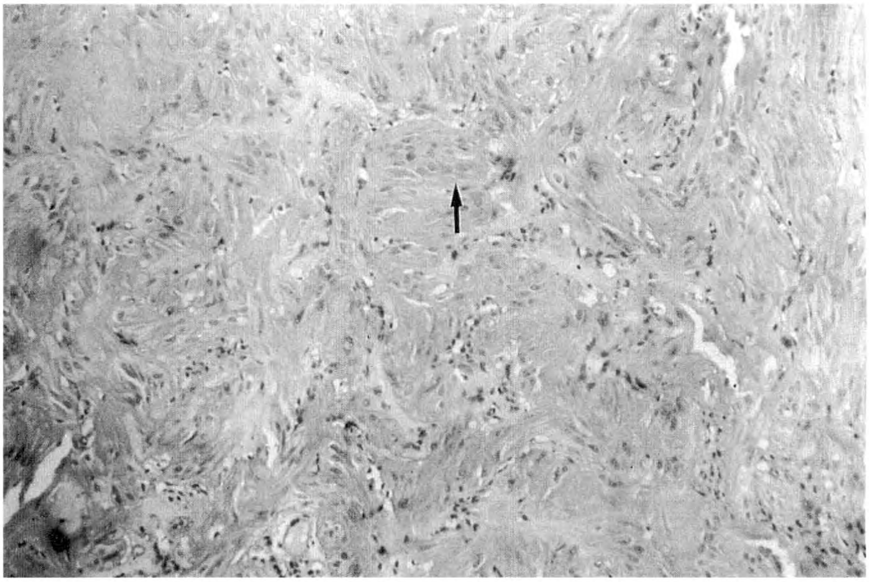
(FNAB) was performed at another institution, and the lesion was initially diagnosed as suspected papillary carcinoma. Scintigraphy of the thyroid showed that the nodule was cold. One of the authors (P.B.) had the opportunity to examine the slides, which were characterized by the presence of amorphous or fibrillated hyaline material associated with oval-spindle tumor cells with ill-defined cytoplasm and large nuclei sometimes demonstrating pseudoinclusions. The author performed a second FNAB, which yielded similar findings (Fig 1); in addition, Congo red stain for amyloid was negative and immunostaining was positive for thyroglobulin and negative for thyrocalcitonin. Hyalinizing trabecular adenoma was diagnosed, and histologic evaluation of the surgical specimen confirmed said diagnosis.

Case 2. A 43-year-old woman had a cold, hypoechoic, nonpalpable nodule in the right lobe of the thyroid that was noted on thyroid scan and scintigraphy. An echo-guided FNAB was performed. The slides, obtained with 2 passes, were composed of abundant material characterized by fragments of sclerotic and hyaline tissue associated with elongated-ovoid epithelial cells with oval nuclei often demonstrating pseudoinclusions (Fig 2). A diagnosis of HTA of the thyroid was established and confirmed on histologic examination of the surgical specimen (Fig 3).

DISCUSSION

Hyalinizing trabecular adenoma is an uncommon benign lesion of the thyroid described in 1982 by Ward et al[1] as "hyaline cell tumor of the thyroid with massive accumulation of cytoplasmic microfilaments," but first named as HTA in a report from Carney et al.[2] The lesion generally occurs within a nor-

Fig 3. (Case 2) Histologic aspect of lesion in Fig 2 (H & E, original ×100). Hyalinizing trabecular adenoma of thyroid demonstrates rich stromal component in which epithelial cells lie in parallel array (arrow), here and there developing storiform pattern.



mal thyroid gland, but findings of HTA have also been reported in multinodular goiter and thyroiditis.[10,11] It has a characteristic female predilection. Macroscopically, HTA presents as a solid, nodular, circumscribed or encapsulated lesion 0.3 to 4 cm in size. Histologically, the tumor has a palisading growth pattern arranged in trabeculae or nets of polygonal, oval, or spindle cells vertically inserted in capillaries and embedded in a hyaline stroma. Basement membrane overproduction, demonstrated by antibodies for type IV collagen and laminin, seems to form the hyaline stroma.[12] Calcification of this extracellular matrix resembles psammoma bodies. The epithelial cells adjacent to the hyaline stroma may show mummification. Pseudofollicles or true follicles with colloid amyloidlike material are seen in the intertrabecular spaces. The neoplastic cells have eosinophilic granular cytoplasm and oval or spindle nuclei. Perinucleolar clearing, nuclear grooves, and intranuclear pseudoinclusions are observed.[13,14] Vascularization is detectable. Mitoses are rare.[2]

In some areas, the cells may be organized into clusters like the classic *Zellballen* of paraganglioma.[4] Because of this histologic feature, the term paragangliomalike adenoma was introduced to indicate HTA of the thyroid.[15] The distinction between HTA and paraganglioma is more difficult when the *Zellballen*-like pattern is associated with an extremely vascular stroma and when the neoplastic cells stain positively for chromogranin and neuron-specific enolase, 2 distinctive markers indicating a neuroendocrine cellular differentiation. Findings of colloid within follicular structures and thyroglobulin positivity ensure a correct diagnosis,[16] however. Besides, paraganglioma rarely arises in the thyroid gland.[17]

Differential diagnosis should be made with medullary and papillary carcinomas, 2 entities requiring a more aggressive treatment because of their malignant behavior. Both histologic and cytologic features may lead to the misdiagnosis of HTA.[18] The presence of spindle-shaped cells and hyaline stroma is reminiscent of a medullary carcinoma. Congo red negativity, a finding inconsistent with amyloid, and immunohistochemical stains proving negative for secretory products (such as calcitonin) and positive for thyroglobulin rule out the diagnosis of medullary carcinoma,[19] however.

High cellularity, intranuclear pseudoinclusions, nuclear grooving, and psammoma bodies are cytologic features similar to those of papillary carcinoma, but are also not exclusive to both HTA and papillary carcinoma.[20] These findings would generally be expected with a higher rate and a more constant distribution in papillary carcinoma.

It is very difficult to detect HTA correctly on the basis of FNAB alone. The 2 cases presented here are examples of the likelihood of FNAB's being able to diagnose HTA. Both lesions were cytologically and histologically unusual, showing a noninvasive histologic pattern that was more trabecular than papillary or follicular. In addition, areas of sclerosis or hyalinization were detectable in the tumors.

Histologic evaluation and immunohistochemical staining contribute further information for a conclusive diagnosis. Hirokawa et al[21] stated that positive tumoral cell cytoplasm immunostaining for MIB1 should be considered a characteristic feature of HTA. In a recent paper, the finding of cytoplasmic yellow bodies, characterized by lipofuscin, in a review of 75 hematoxylin and eosin–stained sections of HTA, was considered an additional distinctive feature for the diagnosis of the tumor.[22]

Though the benign behavior of HTA is generally accepted, signs of malignancy such as vascular and capsular invasion have been reported,[6-9] suggesting that HTA should be considered not as a distinct lesion, but as a morphological pattern observed in several thyroid diseases.[23] In particular, Fonseca et al[5] considered HTA as a trabecular variant of papillary carcinoma because of its similar nuclear characteristics, the occurrence of both lesions in the same patient, and the coexistence of the histologic features of HTA and papillary carcinoma in the same primary and metastatic tumoral masses. The finding in HTA of stratified epithelial-type cytokeratins that are commonly present in papillary carcinoma further supports the relationship between HTA and papillary carcinoma. However, further investigations and a much longer follow-up are advisable in ascertaining the potential malignancy of HTA.

In the 2 presented cases, both of which underwent thyroid lobectomy, no other malignant tumoral lesions were found in the surgical specimens or in the glandular parenchyma outside the nodular lesions.

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VERTIGO: ANALYSIS BY MAGNETIC RESONANCE IMAGING AND ANGIOGRAPHY

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The relationship of vascular disease of the vertebrobasilar artery system to isolated vertigo was examined by magnetic resonance imaging and angiography. Eighty-nine individuals complaining of vertigo were evaluated by standard otoneurologic investigations, and the data were correlated with the vascular patterns of the cervical region and posterior fossa. The age distribution extended from the fourth decade to the ninth decade; the peak occurrence was observed in the eighth. Approximately 85% of the group experienced numerous episodes of vestibular dysfunction from months to years before examination; the remaining segment was examined following the first episode due to severity or persistence of symptoms. The criteria for vascular abnormality proposed by the authors are based upon comparison with previous normal findings. Approximately 52% of the cohort demonstrated abnormal configurations or evidence of diminished flow within the vertebrobasilar artery system. Of this segment, a vertebral artery was most frequently abnormal, in 76%; the basilar artery was judged pathological in 32%, and combined disease of several arteries was evident in 20%.

KEY WORDS — magnetic resonance angiography, magnetic resonance imaging, vertebrobasilar artery system, vertigo.

INTRODUCTION

The relationship of arterial disease in the brain stem and cervical region to vertigo is the subject of this discussion. This investigation includes an examination of the regional arterial vasculature and the brain by magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA). The abnormalities and variations from the normal configurations[1,2] that are identified in this study are considered to be potential or clinically confirmed sources of vestibular disease. Images depicting diminished flow due to partial occlusion, various degrees of stenosis, or total obstruction are examined, and the impact upon the vestibular system is discussed.

The authors recognize the numerous factors that cause vertigo, such as cerebellar or medullary neoplasm, or which are associated with this symptom, for example, Meniere's disease or multiple sclerosis. But there are many individuals with vertigo in whom, following an exhaustive survey, the underlying etiologic factors are not conclusively defined.

We surveyed a group of vertiginous individuals in whom the etiopathology was unclear. Vascular imaging (MRA) was employed with the specific goal of examining the circulation of the posterior fossa; we viewed the normal and abnormal images, flow volumes, morphology, and the potential relationship to this form of dysequilibrium. We recognize and stipulate that the morphology of the vertebrobasilar artery (VBA) system and the associated branches is quite variable, contingent upon the developmental anatomy, collateral connections with overlap of perfusion, watershed regions, and the superimposed intrinsic diseases of atherosclerosis, ectasia, and thrombosis. Greater detail concerning these factors follows in the subsequent text.

VASCULAR IMAGING AND PATHOLOGY

Disorders of the large intracranial and brain stem arteries, ie, the macrovasculature, may be readily identified by contrast angiography,[1,2] MRI, MRA,[2-4] and ultrasonography.[5,6] The radiographic criteria for intracranial atherosclerotic vascular disease include luminal irregularities and stenoses, elongated tortuous vessels, and fusiform aneurysm. The diseased basilar artery (BA) may assume an ectatic or elongated configuration with resulting compression upon the posterior aspect of the third ventricle or foramen of Monroe.[4] The pathophysiology, regional metabolic disturbances, and resulting abnormal morphological configurations have been described.[7,8]

The consequence of a major regional vascular occlusion is well demonstrated by MRI. The localized infarction and the damage of neural structures evolve over months to years and appear as regional encephalomalacia or a focal hyperintense area.[2,3] The complementary MRA may define the specific vessel and the site of arterial occlusion to the medium vessel level. By contrast, disease of the small vessels, ie, microvasculature, such as the distal branches or terminal arborizations of the regional arteries, is not directly visualized in the angiographic images, due to insufficient resolution of the technique, though the pattern of dam-

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age may include small hyperintense areas or lacunar infarcts within the brain, cerebellum, or brain stem.

The normal arterial anatomy of the posterior fossa is inconsistent; the perfused areas relating to a named artery, for example, the posterior inferior cerebellar artery, may be quite variable, with developmental overlap into adjacent regions in the cerebellum and brain stem.

The primary goal of the authors is to identify these morphological characteristics or patterns that may be associated with cerebral hypoperfusion leading to vertigo.

The authors initially evaluated the entire VBA system, but the status of the vertebral artery (VA) and BA received the highest priority for examination, due to numerous anatomic inconsistencies and variation in the peripheral divisions.

Once this investigation was under way, we recognized the need to establish adequate criteria for normal VBA anatomy. The normal controls include 20 individuals who were assessed for nonvertiginous complaints such as cephalgia, migraine, tic douloureux, tremor, and acoustic neuroma. The MRA was carried out after the MRI as a supplemental study, primarily to examine the normal vasculature for the purpose of comparison with potential abnormalities. The normal segment of subjects, therefore, was defined as free of vertigo and with a normal MRI; the MRA was then examined and defined with respect to the entire group. Following integration of the data, we determined the following observations from this group of individuals extending from the teenage years to the eighth decade. First, the cerebellar branches could be identified by MRA and the point of origin in approximately 25% of cases. The distribution of the vessels in the peripheral collateralization across the cerebellar cortex could be visualized occasionally, but the terminal arborization was generally attenuated to a narrow diameter, ie, less than 0.6 mm, which defied identification. Second, the VAs were widely patent, representing adequacy of volumetric flow. A dominance or imbalance in diameter was noted in accord with previous reports.[2,3] Each normal subject had paired and patent VAs, and there was no evidence of stenosis. Tortuosity or a kinking with 2 or fewer angulations was seen in 2 of the older subjects, but nonetheless, the lumens were adequate. Third, the BA assumed variable configurations: straight in approximately 50% of individuals, mildly curved in 30%, and sinusoidal in 20%. These findings were noted in each age group, including the teenaged subject, and are therefore considered developmental variants. The diameters of the BA were within the normal range. Fourth, the MRI findings in each of these subjects were normal except for the consequences of the aging process, with due allowance for the common findings of cortical shrinkage and the microvascular damage due to local ischemia uniquely noted in the elderly.

LOCATIONS OF VERTIGO-PRODUCING LESIONS

The initial problem in the evaluation of vertigo is localization of the lesion; the second concern is the cause of the vestibular disorders. These issues are examined insofar as a vascular component can be considered causative; other factors, including idiopathic, traumatic, and viral entities, are not entertained in this study, and cases considered to be related to these factors are eliminated from analysis. From a clinical standpoint, the distinction between a peripheral, ie, labyrinthine, source versus a central disorder is often not clear. For example, the combined symptoms of vertigo, unsteady gait, and nystagmus are in many instances considered labyrinthine in origin, when in fact, subsequent evidence confirms a lesional site in the medulla or cerebellum.

Grad and Baloh[8] reviewed 42 individuals complaining of isolated episodes of vertigo. Longitudinal investigation revealed 12 of the 42 individuals to have symptoms of VBA insufficiency characterized by isolated episodes of vertigo, and they subsequently developed an infarction. The occluded sites were anterior inferior cerebellar artery (2 subjects), cerebellar (otherwise unclassified; 4), labyrinthine (1), and multifocal (5). They concluded that the vestibular labyrinth could be vulnerable to focal ischemia of the internal auditory artery. In addition, they emphasized the difficulty of localizing a lesion within the central nervous system when the symptoms are limited to episodes of isolated vertigo. Baloh[9] later commented on the improved diagnostic capability of modern imaging techniques employed in the investigation of vertigo, and the complexity of the differential diagnosis between brain stem and cerebellar strokes and peripheral vestibular disorders was further emphasized.

Huang and Yu[10] described 6 individuals with vertiginous complaints who demonstrated lesions in the cerebellum on computed tomography. As a result of these data, the authors suggested that caution be observed in the evaluation and management of vertiginous individuals and that a central localization not be overlooked.

Yamasoba et al[11] analyzed lacunar infarcts identified by MRA in individuals over 60 years of age. The preponderance of vascular occlusive findings was noted in the brain stem (17 of 48 patients) and posterior cranial fossa (26 of 48). Abnormal otoneurologic evidence from electronystagmography (ENG) correlated with the MRI, primarily in lesions within the middle and lower pons and upper medulla. In many instances, the vestibular symptoms corresponded with those of a peripheral labyrinthine disorder.

The authors have previously described the syndromal vertigo complex and correlated midbrain, pontomedullary region, and cerebellar abnormalities by MRI and MRA.[12] The radiographic evidence of regional infarction and vascular occlusive disease accurately established the location of damage. We emphasized in that report that multifocal disease may concurrently impact upon other areas that similarly can produce symptoms of dysequilibrium, such as cerebellar ischemia and, potentially, hypoperfusion of the temporal lobe, the cortical terminus of the vestibular apparatus.

PERFUSION AND HEMODYNAMIC FACTORS

Since the disorders of perfusion relate to many factors, including the channel lumen, pressure, and flow, a brief review of the pertinent hydraulics follows. This information is essential to the understanding of incomplete or total arterial occlusion, with the sequelae of recoverable neural structures in the former, such as transient ischemic attacks, and irreversible damage in the latter, with permanent functional disturbance and anatomic destruction. This material is particularly relevant to the interpretation of ultrasonography and activated vascular flow in MRA. There are 3 major concepts: 1) principles of hydraulic flow and pressure; 2) anatomic factors, ie, arterial anatomy; and 3) quantitative data of specific arterial volumetric flow.

These factors, in turn, impact upon the capacity of the VBA system to perfuse the vestibular areas subserving balance, and result in a symptom of disorder, eg, vertigo. Application of this information to cerebral ischemia considers 1) the pathological decrement of flow volume; 2) the duration of the perfusion deficit; 3) the location of the lesion and relevance to function; and 4) the compromised tissue volume. Extensive information has been derived from carotid artery studies and is referenced as needed, but the major application of this material is to the VBA system.

Physical Principles. The significant parameters that influence flow volume through a vessel or artery include 1) cross-sectional area; 2) steady state of velocity; 3) constant hydrodynamic pressure (ie, blood pressure); and 4) autoregulation, which modifies the arterial caliber in relation to the reduction of force (ie, variable blood pressure); this factor is relatively small, ie, less than 5%.[13]

The fundamental equations for flow in a tube include the Hagen-Poiseuille law[14] and Reynolds' and Bernoulli's principles.[15] The Hagen-Poiseuille law evaluates flow in a fixed tube and states that a positive effect results from an increase in radius and pressure, while a negative or diminished flow relates to an increased length and viscosity of the fluid. This principle also describes a quadratic relationship of the radius to flow and requires a steady state within the system. Reynolds' number relates to the viscosity, velocity, and diameter of a tube.[15] In general, turbulence is exaggerated by an increase in the diameter of a tube with a steady flow rate. Bernoulli's law applies to the impact of a smooth-walled stenotic area on flow and pressure. Initially, the volumetric flow rates are identical on both sides of the narrowed segment. In addition, the pressures proximal and distal to a narrowed area are equal until the stenosis reaches such significance as to impact negatively on the volume through the narrowed area. As a sequel, turbulence develops due to the nonlaminar flow associated with vortices and the uneven reversal of flow at the lateral margins of the stream.

General Patterns of Flow. The conventional scheme of cerebral blood flow divides the anterior circulation from the internal carotid arteries and the posteriorly derived flow through the vertebral system. The anterior cerebral flow accounts for approximately 82% of the total, and the residual 18% is from the posterior sources. Collateralization, retrograde flow, and bilateral connections through the communicating arteries may compensate for regional arterial disease.[14]

Specific Flow Volumes. Hemodynamic modifications secondary to arterial stenosis are identified by transcranial sonography.[5] The rate of flow velocity may increase after a diameter reduction of about 40%. Downstream (poststenosis) turbulence is evident in the parastenotic area associated with diminished flow velocity and decreased pulsatility. On the proximal side (prestenosis), reduced flow velocity and increased pulsatility result from increased peripheral resistance.

Flow data derived from ultrasound were reported by Boyajian et al.[14] The data from cross-sectional measurements indicate that the carotid arteries make up 67% of the total vascular area. The flow volumes through the individual cervical vessels were derived from ultrasonic studies; the data are presented in Table 1.

Asymmetry of flow of greater than 50% was noted in the anterior circulation in 70% and in the posteri-

TABLE 1. FLOW VOLUME AND ARTERIAL DIAMETER

Artery	*Mean Diameter (mm)*	*Mean Flow (mL/min)*
R ICA	5.5 (0.72)	147 (35)
L ICA	5.8 (0.71)	143 (29)
R VA	4.0 (0.64)	34 (16)
L VA	3.9 (0.73)	32 (12)

Numbers in parentheses indicate range of variation.
ICA — internal carotid artery, VA — vertebral artery.

or circulation in 29% of the normal individuals.

The effect of age on volumetric flow was evaluated in 116 normal individuals, age range 16 to 78 years, by Donis et al.[16] From ultrasonic flow analysis of the anterior cerebral circulation, the authors concluded that an age-dependent decline in carotid artery flow occurred.

Mean flow rates and mean peak velocities of the carotid, basilar, and cerebral vessels have been determined from nongated 2-dimensional phase contrast slice techniques. The relevant data from the reports of Turski et al[17] and Marks et al[18] are in Table 2.

Inconstant variation of the lumen may result from atherosclerosis, particularly at the point of origin or termination into other vessels. Variations in blood pressure, ie, non–steady state, relating to cardiac irregularities, postural influence by gravity, and regional compression, impact on the flow with inconsistent and unpredictable results.

MATERIALS AND METHODS

There are 100 individuals in this study; 89 presented with isolated vertigo, occasional nausea and vomiting, and a concomitant gait disturbance. The remaining 11 subjects are considered to have "syndromal vertigo," which is defined as a combination of vestibular disease and objective evidence of cranial nerve or cerebellar dysfunction. This segment of the study was previously reported in detail and is included for comparison with the "isolated vertigo" cohort.[12]

We have eliminated from this study individuals with identified disorders such as Meniere's disease, acoustic neuromas, benign postural positional vertigo, vestibular neuronitis, intracranial diseases (including neoplasm), demyelinating disorders, cardiac arrhythmias, primary hematologic disorders (polycythemia), and emotional disorders.

TABLE 2. ARTERIAL FLOW AND VELOCITY

Artery	*Volumetric Flow Rate (mL/min)*	*Mean Velocity (cm/s)*	*Peak Systolic Velocity (cm/s)*
Basilar[18]	164 ± 12	40 ± 3	51 ± 4
Right carotid[17]	352 ± 21	46 ± 4	63 ± 4
Left carotid[17]	283 ± 12	40 ± 3	51 ± 4

Data are mean ± SD.

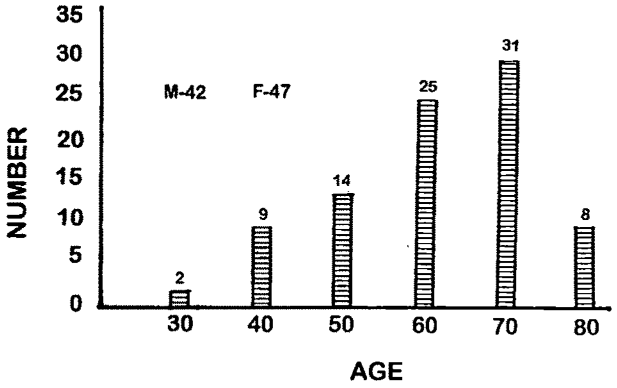

Fig 1. Analysis of vertiginous individuals by age. Gradual increase is observed with seniority, with peak in eighth decade. Male-female ratio is approximately balanced.

As part of the group selection process for the term "isolated vertigo," there was no inclusion in the group of patients with evidence or recollection of hearing loss. A second criterion, visual complaints, ie, ocular dysmetria, blurring, or field cuts, was employed and found historically positive in approximately 25% of the cohort. Syncope, or an unconscious episode, was noted in a small segment (<10%), but total recovery was noted at the point of investigation in each subject.

The symptoms and disability of the vestibular derangement followed a variable course: 85% complained of recurring episodes over weeks to years, and the remaining 15% were surveyed due to the severity and morbidity associated with the initial attack.

Since this was an assessment of the total subjects presenting with the symptom of vertigo, we have not excluded any individual on the basis of age alone. While this study identifies a preponderance of individuals toward the senior measure of life, vertigo does occur in young, adolescent, and middle-aged subjects. Although few in this data base, the young have not subjectively differed to a large degree from the elders. Nonetheless, the disorders of aging, including hypertension, atherosclerosis, and diabetes mellitus, were more common in the senior group.

The age distribution of the entire group extends from the 30s to the 90s; the peak incidence was noted in the eighth decade (Fig 1). The cases are approximately balanced between the sexes (42 men and 47 women). The comprehensive evaluation of the individuals, in addition to radiographic imaging, included audiometry, ENG, and auditory brain stem response.

The latter data will be reported separately and correlated with the magnetic resonance studies.

The techniques of arterial and neural imaging are well described in the current literature. Therefore, for this reason and for spatial limitations, the authors refer to available descriptions of methods for the interested reader.[2-4,19-21]

DIAGNOSTIC CRITERIA

The images were independently reviewed by a neuroradiologist (B.L.) and the other authors (L.W.W., J.J.W.). The criteria for diagnosis and interpretation were established by the combined judgment of the group and are proposed in the subsequent section. The intracerebral status defining the evidence of neural damage secondary to vascular disease is described insofar as it is relevant to this investigation.

The following criteria are based on the investigation of normal individuals, with due consideration of the parameters of anatomic structure and morphological variation.[1]

Vertebral Artery. The normal VA configuration in the young is essentially straight; with aging, a mild tortuosity may develop consequent to hypertension and atherosclerosis. The average configuration varies widely and may progress into a pathological distortion; the radiographic decision between normal and abnormal patterns is relatively subjective and based on the experience of the radiologist and clinicians. Following integration of the combined experience of the authors, we have stipulated the features we suggest to be abnormal.

Criteria for abnormalities of the VA include 1) nonvisualization, 2) stenosis or absence of antegrade flow, 3) difference of diameter in excess of 3:1 by comparison of VAs at similar cervical levels, and 4) tortuosity, ie, corkscrew appearance, consisting of 2 or more bends in the cervical region.

While it is recognized that in the asymptomatic subjects the left VA is usually larger than the right, on review of our data we found that this observation was not significant to the relationship of vertigo and vascular disease. Absence or extreme narrowing of the VA may be considered pathological, and a relatively rare variation in the point of origin is recognized in fewer than 1% of subjects.

Basilar Artery. The BA is extremely variable in contour and position. The most fixed location is in the midline at the bifurcation into the posterior cerebral arteries. While this artery is usually straight in the young, the older age group may display dislocation from the midline and an S configuration in a small percentage, ie, 16%.[22]

Structural abnormalities of the BA include 1) curvature greater than 90% from the midline, 2) stenosis with segmental absence of flow or multiple areas of stenosis presenting a beaded appearance, and 3) aneurysmal dilatation.

We note that the radiographic evidence for total arterial occlusion is diagnostic and conclusive, namely, an area of infarction demonstrated by MRI, and vascular interruption identified by MRA. In clinical practice, however, the interpretation of partial stenosis or abnormal configurations of the vascular patterns may, among several reviewers, be controversial. Nonetheless, the criteria were based on the extreme variations within the vertiginous cohort from the so-called normal patterns derived from asymptomatic individuals.

Combined disease, that is, concomitant VA and BA disorders, was noted in 9 cases (20%) of the vertiginous group. Evidence of generalized vascular disease including the carotid arteries and intracranial vasculature was identified in 18 of the 89 individuals (20%). Cerebral infarctions were described in 10 subjects; these were located in the cortical and subcortical regions without an apparent predilection toward specific intracranial locations.

DATA

Overall, 52% of the individuals in this study (89 total) demonstrated some radiographic evidence of vascular abnormality. These data were then classified according to the involved vessel.

Vertebral Artery. Thirty-one subjects (67% of the abnormal vascular group) had focal disease, including 22 individuals with severe stenosis or nonvisualization. Five subjects displayed moderate to extreme tortuosity, while 2 exhibited dolichoectasia. In addition, there were 2 cases of subclavian steal syndrome with compensatory retrograde flow confirmed by contrast angiography from the contralateral VA (Figs 2 and 3).

Basilar Artery. Abnormalities were identified in 15 cases (33%) that included total occlusion, incomplete stenosis with identifiable runoff into the upstream vessel, and, most commonly, extreme tortuosity (Fig 4).

CASE REPORTS

The following cases are summarized to illustrate the symptoms, physical examination, and correlation with the abnormal vascular configurations.

Case 1. This 74-year-old woman complained of spinning, insecurity of balance, lightheadedness, and diplopia that had persisted for 2.5 years. The findings on neuro-otologic examination were within nor-

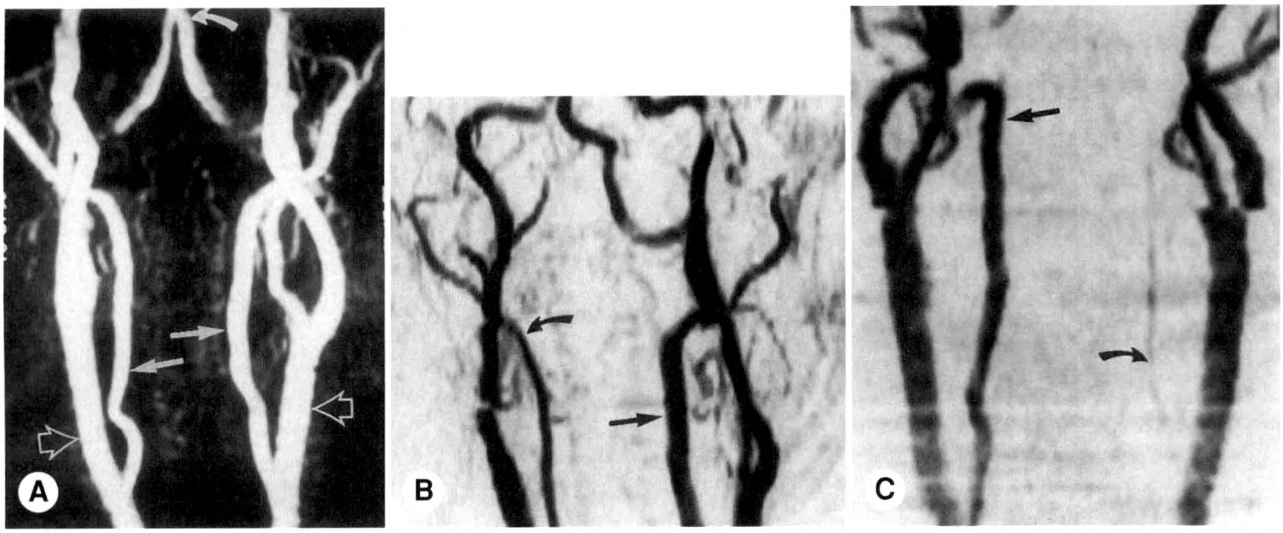

Fig 2. Magnetic resonance angiography (MRA) of cervical region. A) 88-year-old man with normal configuration of vertebral arteries (VAs; straight solid arrows), merging into basilar artery (BA; curved arrow) and common carotid arteries (open arrows). B) 76-year-old woman. Note asymmetry of VAs, with left (straight arrow) approximately 3 times larger than right (curved arrow). C) 66-year-old man. Right VA is patent and reveals straight course below atlas loop (straight arrow). Left VA demonstrates severe stenosis and minimal flow (curved arrow). This vessel was not identified by ultrasonography.

mal limits of age; a presbycusis pattern was evident (speech reception thresholds, 40 dB on right and on left). The gait was satisfactory, though the patient failed tandem testing. Pursuit eye movements were normal, and the caloric responses during ENG were balanced (Fig 3A).

Case 2. This 52-year-old man complained of isolated vertigo occurring suddenly 3 weeks before examination. The episode was acute and described as spinning. There were no complaints of nausea, vomiting, visual disturbance, weakness, or gait disturbance. The findings on neurologic examination were normal at the time of examination. The hearing was compatible with age, ie, presbycusis. A follow-up examination 3 months later disclosed total recovery and no further recurrence of symptoms (Fig 4A).

Case 3. This 68-year-old woman was examined for an acute episode of vertigo occurring 2 months prior to examination and associated with nausea and vomiting. The subsequent recovery had been slow, and the gait was unstable, though she could ambulate without help. The neuro-otological examination findings were normal except for direction-changing nystagmus on positional change. Other results included an essentially balanced caloric response, normal hearing, and a relatively indistinct auditory brain stem response morphology. The MRA revealed marked tortuosity of the BA (Fig 4B).

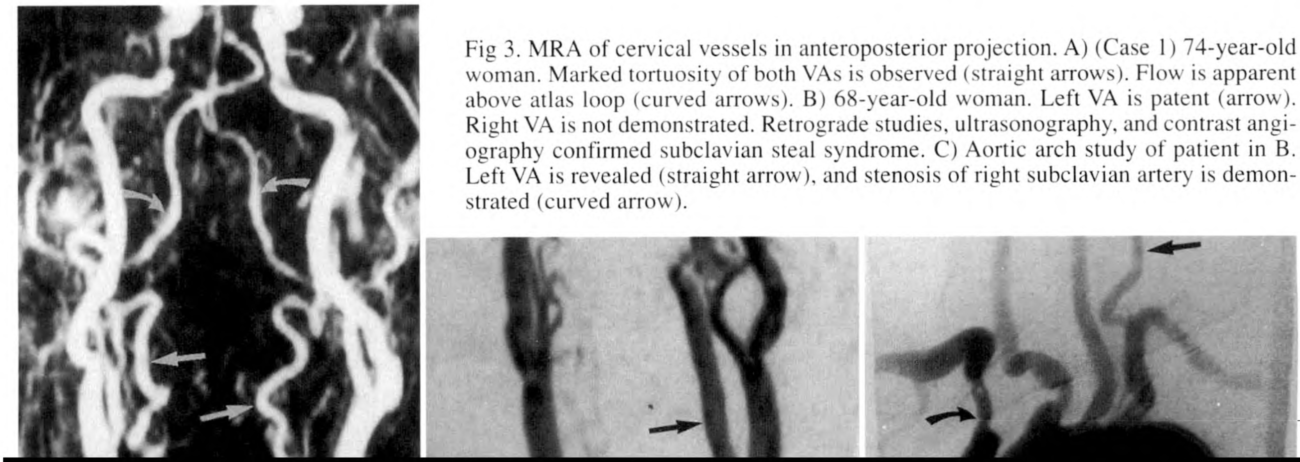

Fig 3. MRA of cervical vessels in anteroposterior projection. A) (Case 1) 74-year-old woman. Marked tortuosity of both VAs is observed (straight arrows). Flow is apparent above atlas loop (curved arrows). B) 68-year-old woman. Left VA is patent (arrow). Right VA is not demonstrated. Retrograde studies, ultrasonography, and contrast angiography confirmed subclavian steal syndrome. C) Aortic arch study of patient in B. Left VA is revealed (straight arrow), and stenosis of right subclavian artery is demonstrated (curved arrow).

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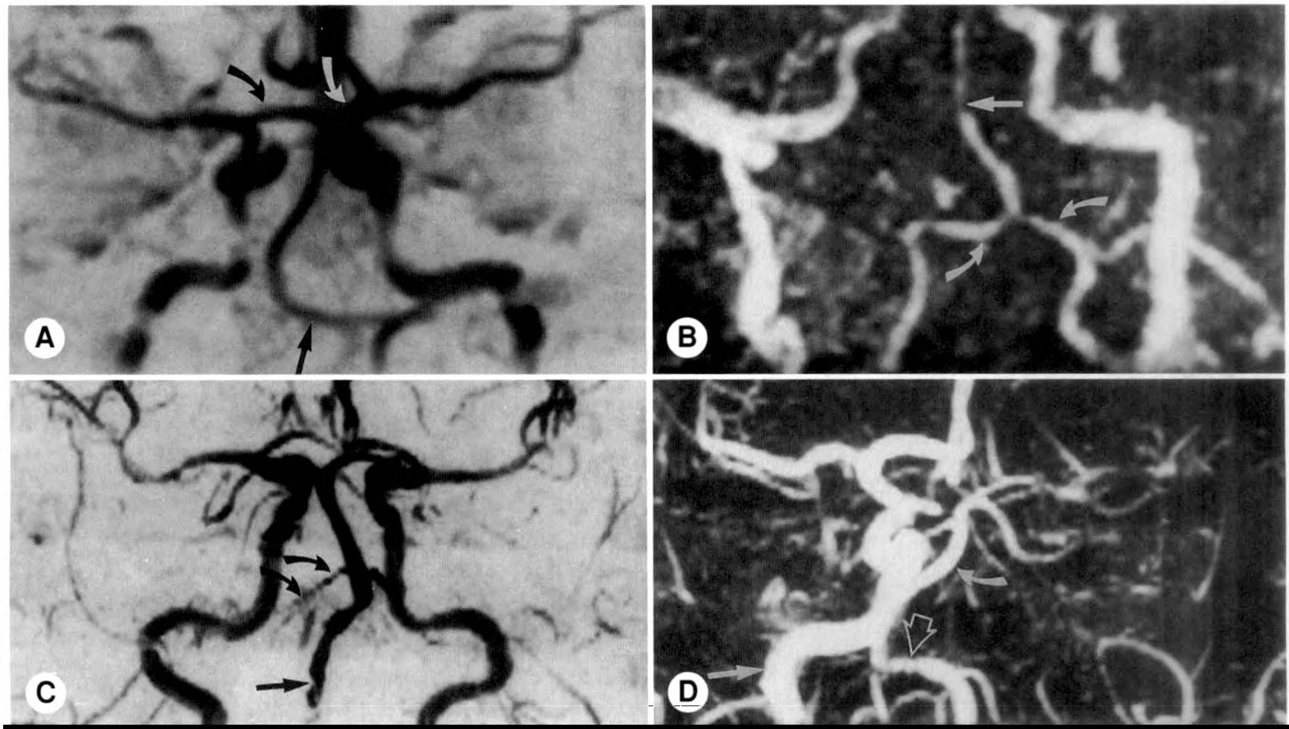

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Introduction. Other considerations, such as the role of the electrophysiological tests of audition, ENG, and electro-oculography, are not within the purview of the current paper.

The radiographic images were classified according to configuration, including tortuosity, deformity, and stenosis (partial or complete) and the relationship to vertigo.

In the older individual, the configurations of tortuosity and ectasia are related to hypertension and atherosclerosis. However, in the younger segment, congenital atresia of a VA, absence of collateralization from the anterior circulation, and incomplete development of the circle of Willis may predispose to symptoms that may be precipitated by positional change and extracranial VA compression.

The evaluation is based on "criteria for vascular abnormalities" that we propose. According to these standards, 52% of the 89 examined subjects displayed grossly apparent aberrations from the normal. The majority of this segment represented VA disease. Less frequently involved, the BA was abnormal in somewhat fewer subjects (33%). Combined VA and BA disease was identified in 9 cases. Generalized or multifocal arterial disease including the extracranial carotid arteries, the anterior cerebral arteries, and the posterior circulation was noted in 20% of the cohort.

As a technical factor, we recognize that there is a quantitative difference between the degrees of stenosis measured by MRA and those measured by invasive contrast angiography. This minor differential (15% or less) is irrelevant to this project, since the authors are primarily concerned with the actual flow volume rather than the computer-derived measurement of patency. Further, a perfusion disorder is the dynamic precursor to the vestibular symptoms related to focal ischemia, whether the stenosis is partial or complete. For example, complementary data derived from ultrasonography and direct flow measurement of carotid artery stenosis suggest that adequate perfusion is maintained through a stenotic area of less than 80% narrowing.[5,23] With greater degrees of stenosis, the resulting damage from circulatory interruption corresponds with the flow volume deficit and the duration of ischemia.

From a pathophysiological perspective, previous studies described the consequences of middle cerebral artery occlusion through the immediate and delayed effects of local tissue damage. Initially, the effects are cytotoxic and include water retention, ie, cellular edema. The delayed injuries are subsequent to vasogenic influences on the regulatory phase, with modification of the blood-brain barrier, cerebral compression, and reactive hyperemia. Additional evidence suggests that decreasing cerebral tissue perfusion below 40% to 30% of normal raises cerebral water content.[24] Other data[25,26] revealed the formation of brain edema when the regional tissue perfusion fell below 10 to 20 mL/100 g per minute. Consequently, a reduction of blood flow below about 20% of normal may cause irreversible brain damage if persisting beyond several hours. The postulated mechanism follows the sequence of an initial vascular-based ischemia, the consequent brain edema, and the final stage of destructive brain ischemia. In this current study, and confirmed by longitudinal historical analysis, some of the individuals displayed progressive cerebellar and/or brain stem symptoms that are now identified by MRI and MRA to be a consequence of ischemic or occlusive disease.

The general principles of volumetric flow that relate to arterial radius, pressure, length of the tube, and viscosity certainly play a part in local perfusion. A modification of the flow may ensue from regional turbulence associated with advanced tortuosity. Consequently, with the additional factors of variable pressure and extrinsic compression, the volumetric flow may intermittently diminish from a marginal to a symptom-producing level of hypoperfusion.

Another consideration is unilateral vertebral stenosis; we suggest that this condition is a consequence of intrinsic vascular disease, ie, atherosclerosis. This hypothesis is premised upon the rareness of congenital stenosis in the young and the inordinately high existence of abnormal individuals in this study, ie, 31 among 89. As a consequence of acquired VA stenosis, the net flow from the residual vessel to the BA may be markedly diminished unless congenital dilatation related to contralateral agenesis (rare) or hypoplasia coexists and compensates for the altered flow.

An additional goal in this study is recognition of central vestibular disease that can be distinguished from peripheral "end organ" disease. The radiographic data confirmed the fact that approximately 50% of the study group revealed essentially normal "large vessel" structures. The authors do not imply that all vertigo equates to vascular insufficiency, but suggest that in a significant segment, identification of a vascular abnormality is defined by MRA. Speculation on other etiologic factors suggests "small vessel disease" not visualized by MRA; congenital variation of the posterior fossa circulation, including collateralization; cervical spondylosis with intermittent vascular compression; and vasospastic disease of the VBA system such as basilar migraine.

From a longitudinal study of vertiginous patients, we observed that many individuals described symptoms that initially incriminated the peripheral labyrinth as the source of the disorder. An examination of the vertiginous episodes — extending from months to years, often initiated during the fourth or fifth decade, continuing with a variable frequency, and with long intervals between attacks — suggests that a "benign form" of vertigo exists. About 80% of this group, with a median age in the seventh decade, fell into this category. We also assume and postulate that in this segment a transient pathophysiological mechanism such as vasospasm or brief extrinsic compression with subsequent total recovery may intervene without localizing signs. Other factors, such as recurring viral labyrinthitis, vestibular neuronitis, neoplasms, and Meniere's disease, were considered and rejected by history or by laboratory or radiographic evidence. As a further consideration, during the period of this study, 5 acoustic neuroma patients were identified and were distinguished by the clinical course and laboratory data from the nonsyndromal patients. The actual ratio of patients with tumors to evaluated vertiginous patients was 1:20 (5%); however, the strict criteria for entry into the analysis precluded a much larger group, reducing the relationship by at least fivefold (ie, 1:100 patients).

On the basis of the radiographic evidence of stenosis and tortuosity and with the correlation of the hydraulic principles for volumetric flow, we suggest that intrinsic vascular disease is the fundamental disorder leading to hypoperfusion. Second, an extrinsic factor, such as localized cervical compression or a pressure disturbance due to alteration of cardiac output, may be the immediate precipitating factor for the acute vestibular derangement. Third, the impact of tortuosity upon acute-angled branches of the BA and the presence of atheromata at the ostia, a recognized site of predilection, may further modify the consequent flow disturbance.

Integration of MRI with subsequent MRA had very limited yield in individuals suffering from isolated vertigo. Since the episodes were brief, without sequelae or objective findings of associated cranial nerve dysfunction, the MRI findings were rarely abnormal, except for the evidence of preexistent vascular occlusive disease. Indeed, if the etiologic factor for vertigo is a transient ischemic event, the duration of hypoperfusion is brief, and the area of functional anatomic disturbance is limited, a permanent radiographic change will not be identified subsequent to this event. Conversely, in the syndromal vertigo series, wherein a defined multi–cranial nerve or cerebellar disability is apparent due to vascular occlusion (embolic phenomena or thrombosis) rather than a neoplasm, a cerebellar or brain stem defect becomes radiographically identifiable. The dichotomy of peripheral versus central etiologic factors producing vertigo, while traditional, has a limited application to the patients in this study. Since the peripheral lesion is categorized by damage to the cochlea with resultant hearing loss and vestibular dysfunction, vertigo without hearing loss loses the specificity of localization. Positionally induced vertigo and nystagmus, while classified potentially as a peripheral disorder, may in fact reflect cerebellar or brain stem disease. A longitudinal examination tends to clarify the pathophysiology by the acquisition of central findings compatible with an intra-axial disorder. The central subset of vertiginous individuals suffering isolated vertigo at this time may undergo an extensive investigation without a site of lesion confirmed by either MRI or MRA.

We anticipate further improvement in radiographic technology that may resolve the dilemma of central versus peripheral labyrinthine dysfunction.

Finally, as confirmed by this series of cases, MRA is the ideal noninvasive technique for identification of perfusion disorders that in turn allows the clinician a rational basis for a therapeutic program or surgical remediation.

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VALIDATION OF OUTCOMES SURVEY FOR ADULTS WITH CHRONIC SUPPURATIVE OTITIS MEDIA

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Currently, there is no valid, disease-specific outcomes measure to evaluate health impact and treatment effectiveness for patients with chronic suppurative otitis media (CSOM). The Chronic Ear Survey (CES) is a new, disease-specific outcomes measure for CSOM that was administered in a prospective manner to 91 patients with CSOM. It was then validated according to established criteria for reliability, validity, and sensitivity to clinical change by correlation with objective data and self-assessment questionnaires such as the Hearing Handicap Inventory for Adults (HHIA) and the generic 36-Item Short-Form Health Survey (SF-36). Significant correlations between subscale scores of the CES and audiometric data and between subscale scores of the HHIA and SF-36 were found. The standardized response mean for the CES total score was 0.42, indicating moderate sensitivity to clinical change. Overall, results demonstrated that the CES is a reliable and valid instrument for investigation of health status and health-related quality-of-life outcomes.

KEY WORDS — chronic suppurative otitis media, health status, outcome, quality of life.

INTRODUCTION

Comprehensive assessment of the health impact and outcomes of treatment for chronic suppurative otitis media (CSOM) requires a valid and reliable patient-based outcomes measure. Patient-based outcomes measures permit understanding of the effects of disease and intervention on a patient's functioning and well-being from the patient's point of view. Although traditional objective measures such as audiograms, rates of recurrent disease, and complication rates are useful, they are not sufficient for enabling us to understand the total impact of CSOM on a patient. Quality-of-life measures add another dimension to understanding how CSOM affects patients and the benefits as well as trade-offs of various treatments.

Although a few measures for hearing disability have been previously validated, such as the Hearing Handicap Inventory for Adults (HHIA),[1,2] a multi-item, Likert-scaled questionnaire specific to CSOM does not exist at the present time. In addition to creating a quality-of-life outcomes measure for CSOM, we wanted to validate it according to established criteria for reliability, validity, and sensitivity to clinical change. The purpose of this study was to develop and validate a disease-specific outcomes measure for CSOM, which we have termed the Chronic Ear Survey (CES).

METHOD

CONSTRUCTION OF CES

An expert panel was used to develop item areas for CSOM. The initial questionnaires were piloted with open-ended responses. Actual survey items were constructed by use of Likert scaling techniques. The CES (Table 1) contains 13 items that evaluate the frequency, duration, or severity of problems associated with CSOM on a Likert scale with 5 to 6 response categories. The CES generates a total score and 3 domain or subscale scores. The activity restriction (AR) subscale reflects limitations in physical or social activities directly related to CSOM. The 2 remaining subscales are reflective of patient symptoms (ST) and medical resource (MR) utilization.

STUDY POPULATION

Ninety-one consecutive patients with a diagnosis of CSOM participated. Chronic suppurative otitis media was defined as a condition of the middle ear of at least 3 months' duration characterized by irreversible pathological changes in the mucosa of the middle ear or mastoid and associated with constant or intermittent discharge of bacterial origin. Diagnosis was

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TABLE 1. CHRONIC EAR SURVEY

Activity restriction–based subscale

a1. Because of your ear problem, you don't swim or shower without protecting your ear.
definitely true   true   don't know   false   definitely false

a2. At the present time, how severe a limitation is the necessity to keep water out of your ears?
very severe   severe   moderate   mild   very mild   none

a3. In the past four weeks, has your ear problem interfered with your social activities with friends, family or groups?
all of the time   most of the time   a good bit of the time   some of the time
a little of the time   none of the time

Symptom subscale

s1. Your hearing loss is:
very severe   severe   moderate   mild   very mild   none

s2. Drainage from your ear is:
very severe   severe   moderate   mild   very mild   none

s3. Pain from your ear is:
very severe   severe   moderate   mild   very mild   none

s4. Odor from your ear is very bothersome to you and/or others:
definitely true   true   don't know   definitely false

s5. The hearing loss in your affected ear bothers you:
all of the time   most of the time   a good bit of the time   some of the time
a little of the time   none of the time

s6. In the past 6 months, please estimate the frequency that your affected ear has drained:
constantly   5 or more times, but not constantly   3-4 times   1-2 times   not at all

s7. The odor from your affected ear bothers you and/or others:
all of the time   most of the time   a good bit of the time   some of the time
a little of the time   none of the time

Medical resource subscale

m1. In the past 6 months, how many separate times have you visited your doctor, specifically about your ear problem?
more than 6 times   5-6 times   3-4 times   1-2 times   none

m2. In the past 6 months, how many separate times have you used oral antibiotics to treat your ear infection?
more than 6 times   5-6 times   3-4 times   1-2 times   none

m3. In the past 6 months, how many separate times have ear drops been necessary to treat your ear condition?
more than 6 times   5-6 times   3-4 times   1-2 times   none

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based on symptoms consistent with CSOM (eg, purulent otorrhea, hearing loss, etc) and otoscopic evaluation demonstrating 1 or more of the following: cholesteatoma, perforation of the tympanic membrane, or severe retraction of the tympanic membrane. Patients underwent audiometry consisting of a determination of binaural air and bone conduction thresholds and word discrimination scores.

SURVEY FORMS

At entry into the study, all patients received the self-administered CES, the HHIA,[1,2] and the Medical Outcome Study 36-Item Short-Form Health Survey (SF-36).[3,4] The HHIA is a research-validated, 25-item self-assessment questionnaire that is divided into a 13-item emotional subscale and a 12-item hearing handicap subscale. Scores are tabulated per published algorithms from 100 (worst) to 0 (best). The SF-36 is a widely used, research-validated, generic quality-of-life measure with 8 domains of general health, including physical functioning limitations, role functioning–physical problems, bodily pain, general health, vitality, social functioning, role functioning–emotional problems, and mental health. Scores are tabulated per published algorithms from 0 (worst) to 100 (best). Permission to use the HHIA was obtained. Permission to use the SF-36 was obtained from the Medical Outcomes Trust of Boston.

RELIABILITY OF CES

Fifty-two patients with unilateral CSOM and without known interval clinical change from treatment were retested with the CES in the same manner after a 2- to 4-week interval to determine test-retest reliability. These patients were awaiting surgical scheduling, and stability of the clinical condition was confirmed on the day of surgery. Test-retest reliability was calculated via Spearman rank order correlation coefficients for individual items and for subscale and total survey scores. Internal consistency of the CES

TABLE 2. SPEARMAN CORRELATIONS FOR TEST-RETEST RELIABILITY OF CHRONIC EAR SURVEY

	No.	*Spearman r*	*p*
Individual items			
a1	52	.86	<.00001
a2	52	.68	<.00001
a3	52	.74	<.00001
s1	52	.75	<.00001
s2	52	.81	<.00001
s3	51	.83	<.00001
s4	51	.90	<.00001
s5	52	.68	<.00001
s6	52	.81	<.00001
s7	52	.77	<.00001
m1	52	.73	<.00001
m2	52	.80	<.00001
m3	52	.74	<.00001
Subscale			
AR	51	.81	<.00001
ST	50	.91	<.00001
MR	52	.81	<.00001
Total survey	49	.91	<.00001

AR (activity restriction subscale) — items a1, a2, a3; ST (symptom subscale) — items s1, s2, s3, s4, s5, s6, s7; MR (medical resource subscale) — items m1, m2, m3; total survey — items a1-a3, s1-s7, and m1-m3.

was calculated via Cronbach's α correlation coefficients.

VALIDITY OF CES

In addition to its face validity, the CES was assessed for convergent validity through correlations to concurrent audiometric evaluations and by comparison to convergence and divergence scores on previously validated measures, the HHIA and the SF-36. A total of 39 of 91 patients were identified who fit the following criteria: 1) unilateral CSOM, 2) unilateral conductive hearing loss and bone conduction thresholds of <35 dB in the affected ear, and 3) no major comorbid systemic disease.

RESPONSIVENESS OF CES

Instrument responsiveness or sensitivity to clinical change was assessed in the following manner. Of 39 patients used in the validity analysis, 33 patients with successful control of infection and/or hearing restoration, as noted by their otologists in postoperative evaluations, were retested with the CES 6 months after surgery. Longitudinal sensitivity to clinical change was calculated as the standardized response mean, according to methodology described by Liang et al.[5]

STATISTICAL ANALYSIS

All CES data were stored and analyzed with the STATA software package (Stata Corporation, Texas, 1996), which permitted extensive analysis, including complete correlation comparisons between all scores (total scores, subscale scores, and singular item scores) derived on the CES, HHIA, and SF-36. In addition, SF-36 subscale score means were compared to published normative data with Student's *t*-test.

RESULTS

STUDY POPULATION

Fifty-two patients who received the CES were retested 2 to 4 weeks later. A high (96%) retest response rate was obtained. Thirty-nine preoperative patients with complete audiometric evaluations completed the CES, HHIA, and SF-36. The patient population was 64% right-sided and 36% left-sided. Cholesteatoma was reported in 59% of patients. Thirty patients out of 33 who underwent surgery returned the surveys mailed to them, for a 91% return rate.

RELIABILITY OF CES

Test-Retest Reliability. Mean test and retest total scores varied from 43.2 ± 21.5 on the first administration to 43.2 ± 24.7 on the second administration. The test-retest (performance consistency) correlation coefficients among individual items and grouped items were high (Table 2). For example, the test-retest correlation coefficients for AR, ST, MR, and total survey scores were .81, .91, .81, and .91, respectively.

Internal Consistency. The AR ($r = .60, p < .00001$), ST ($r = .86, p < .00001$), and MR ($r = .66, p < .00001$) subscale scores all correlated well with the total survey score. Cronbach's α correlation coefficients (for internal consistency) were calculated as .62 for the

TABLE 3. SPEARMAN CORRELATIONS BETWEEN CHRONIC EAR SURVEY AND AUDIOMETRIC DATA

	s1, s5		*Activity Restriction*		*Symptom*		*Medical Resource*		*Total Survey*	
Audiometric Variables	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
WPTA	.48	.002	–.11	NS	.33	.049	.18	NS	.15	NS
WWRS	–.22	NS	–.06	NS	–.11	NS	.08	NS	–.06	NS
BPTA	.19	NS	.02	NS	.11	NS	.08	NS	.11	NS
BWRS	–.32	NS	–.07	NS	–.18	NS	–.17	NS	–.18	NS

s1, s5 — CES hearing-related items s1 and s5; WPTA — worse ear pure tone average; WWRS — worse ear word recognition score; BPTA — better ear pure tone average; BWRS — better ear word recognition score; NS — not significant.

TABLE 4. SPEARMAN CORRELATIONS BETWEEN CHRONIC EAR SURVEY AND HEARING HANDICAP INVENTORY FOR ADULTS

	Activity Restriction		*Symptom*		*Medical Resource*		*Total Survey*	
HHIA	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Social	.67	<.00001	.38	.0166	.31	NS	.55	.0004
Emotional	.64	<.00001	.43	.0077	.18	NS	.50	.0017
Total	.69	<.00001	.42	.0088	.22	NS	.54	.0005

Social — HHIA social subscale; Emotional — HHIA emotional subscale; Total — HHIA total survey; NS — not significant.

AR subscale, .80 for the ST subscale, .75 for the MR subscale, and .83 for the total survey.

VALIDITY OF CES

Correlation With Audiometric Data. The correlations between the CES and the audiometric variables are shown in Table 3. In addition, 4 elements — speech-level pure tone average in the worse hearing ear (WPTA, mean 38.0 ± 17.1, range 15 to 73), word recognition score in the worse hearing ear (WWRS, mean 90.1 ± 15.4, range 4 to 92), speech-level pure tone average in the better hearing ear (BPTA, mean 15.4 ± 8.8, range 0 to 35), and word recognition score in the better hearing ear (BWRS, mean 94.3 ± 2.3, range 82 to 92) — were collected from patients' audiometric data. (Regarding the ranges for WWRS and BWRS, 92% was arbitrarily assigned as the highest word recognition score, because scores equal to or higher than 92% were regarded as normal, and not further specified in our institution.) The frequencies used for generating the speech-level pure tone average were 500, 1,000, and 2,000 Hz, and W22 test lists were used. The 4 elements were found to correlate with the AR subscale score (mean 12.4 ± 6.0), ST subscale score (mean 22.2 ± 12.5), MR subscale score (mean 8.7 ± 6.1), and total score (mean 42.7 ± 19.9) on the CES. The hearing-related items (s1, s5; see Table 1) were found to correlate significantly with the WPTA ($r = .48$, $p = .002$) and BWRS ($r = -.32$, $p = .05$).

Correlation With Hearing Handicap Inventory for Adults. The correlations between the CES and HHIA subscale scores are shown in Table 4. Additionally, hearing-related items (s1, s5) together correlated significantly with the HHIA emotional subscale score ($r = .53$, $p = .0005$), social subscale score ($r = .58$, $p = .0001$), and total score ($r = .57$, $p = .0002$). Similarly, the social-related item (a3; see Table 1) of the CES correlated strongly with the HHIA social subscale score ($r = .63$, $p < .00001$), emotional subscale score ($r = .69$, $p < .00001$), and total score ($r = .68$, $p < .00001$). The correlations between the HHIA and the audiometric variables were also obtained. The BWRS correlated reasonably with the HHIA social subscale score ($r = -.35$, $p = .03$) and emotional subscale score ($r = -.33$, $p = .05$). Last, a fair, but not significant, correlation ($r = -.32$, $p = .06$) was found between the BWRS and HHIA total scores.

TABLE 5. EIGHT SUBSCALES OF SF-36 GENERAL HEALTH MEASURE

Subscale	*Definition*
Physical functioning (PF)	Limitations on physical activities such as walking, bathing, and strenuous sports
Role functioning–physical (RP)	Problems with work or other daily activities as result of physical health
Bodily pain (BP)	Intensity of bodily pain or limitation due to pain
General health (GH)	Perception of current health and health outlook
Vitality (VT)	Level of energy
Social functioning (SF)	Extent health interferes with normal social activities
Role functioning–emotional (RE)	Problems with daily activities as result of emotional issues
Mental health (MH)	Mental health screening

Correlation With 36-Item Short-Form Health Survey. Subscale scores for the SF-36 were tabulated according to published algorithms.[4] Representing different aspects of general health, the SF-36 is divided into 8 subscales: physical functioning limitations (PF), role functioning–physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role functioning–emotional problems (RE), and mental health (MH) (Table 5).

The 8 subscale scores of the SF-36 were compared with the AR, ST, and MR subscale scores and the total score on the CES, and correlation coefficients were derived (Table 6). The CES subscale scores and total score all demonstrated a significant correlation with the BP and SF subscale scores of the SF-36. The CES total score correlated well with subscales

TABLE 6. INTERSURVEY SPEARMAN CORRELATIONS BETWEEN CHRONIC EAR SURVEY AND SF-36

	Activity Restriction		*Symptom*		*Medical Resource*		*Total Survey*	
SF-36	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
PF	.35	NS	.20	NS	.19	NS	.29	NS
RP	.42	.014	.29	NS	.23	NS	.34	NS
BP	.39	.0234	.54	.0013	.51	.0023	.60	.0002
GH	.27	NS	.33	NS	.31	NS	.37	.0413
VT	.12	NS	.42	.0181	.08	NS	.33	NS
SF	.58	.0006	.41	.0217	.41	.0223	.53	.002
RE	.16	NS	.25	NS	.10	NS	.17	NS
MH	.28	NS	.47	.0073	.13	NS	.42	.0173

PF — physical functioning; RP — role functioning–physical; BP — bodily pain; GH — general health; VT — vitality; SF — social functioning; RE — role functioning–emotional; MH — mental health; NS — not significant.

measuring patients' perception of their general health (r = .37) and mental health (r = .42), whereas the AR and ST subscales correlated well with the RP (r = .42) and VT (r = .42) subscales, respectively. Additionally, there were robust correlations between the social-related item (a3) and the SF subscale (r = .69, p < .00001) and between the pain-related item (s3) and the BP subscale (r = .77, p < .00001).

Responsiveness of CES. The standardized response mean for the CES total score was .42, indicating a moderate responsiveness or sensitivity to clinical change. The CES was thus effective in measuring the positive gains made by the 30 surgery patients studied.

Comparison of CSOM Patients to US Norms. The mean subscale scores on the SF-36 from 33 otherwise healthy CSOM patients were compared with published normative data from the general US population.[6] Although differences were detected between CSOM patients and the reported US normative sample in the SF-36 domains of RP (79.55 ± 37.74 versus 80.96 ± 34.00), BP (72.64 ± 24.56 versus 75.15 ± 23.69), GH (68.23 ± 20.48 versus 71.95 ± 20.34), and MH (72.52 ± 17.55 versus 74.74 ± 18.05), these differences were not statistically significant (p = .63).[6]

DISCUSSION

Chronic suppurative otitis media is a disorder that affects patients in ways that cannot be adequately assessed by laboratory tests or even audiograms. Although the condition is rarely life-threatening, it may have a significant impact on a patient's quality of life. In this study, we introduce the CES as a valid, reliable, and sensitive disease-specific health measure that adds another dimension to our understanding of the impact of the disease on adult patients with CSOM.

The CES demonstrated good test-retest reliability for individual test questions, for all subscales, and for total score. Improvement in wording may further enhance the reliability of some items (a2, s5). The measured test-retest reliabilities for AR, ST, and MR subscales of .81, .91, and .81 and for a total score of .91 were consistently better than those of the well-studied SF-36, which had correlations ranging from .60 to .81 for its subscales when a test-retest method was used.[7] Internal consistency of .83 on the CES was demonstrated by a Cronbach's α correlation coefficient; this met the requirement (.70) for a reliable measure.[8]

The overall comparison between the CES subscale scores and the audiometric data suggests that there is variability in patients' responses to hearing impairment, however mild it might be. Neither a subjective nor an objective hearing test, when used alone, is able to fully capture patients' perceptions of their hearing loss.[1] A correlation of .48 between hearing-related items (s1, s5) and the WPTA, which is higher than the correlations for the usual subjective hearing measures of performance, can perhaps be attributed to the arrangement of 1 side-specific question (s5) in this group. A correlation of –.32 between hearing-related items (s1, s5) and the BWRS explains the sensitivity of the disease-free ear. Hearing in the disease-free ear affects the patient's perception of hearing disability and thus should be included in outcomes evaluation.[9]

The CES is a worthwhile supplement to the standard audiological test battery. The robust correlations between the CES and HHIA suggest that either survey is sufficient to evaluate the perception of hearing impairment. However, the CES accomplishes this with fewer items and, at the same time, evaluates the additional domains of patient symptoms and use of medical resources. The audiometric measures may fail to adequately assess patient-based outcomes that are important to the patients, whereas the information obtained from hearing-related items on the CES enables us to understand patients' hearing complaints in ways that are not captured in traditional audiometric data. These data may facilitate decisions regarding candidacy for surgery, as well as assist measurement of the effectiveness of surgery in regard to hearing improvement.[1] There are several methods, including the "15/30 dB rule of thumb"[9] and the "Glasgow plot,"[10] for evaluation of a patient's benefit from middle ear reconstructive surgery. Comparison of the CES with these outcomes measures is planned.

Correlation of the CES with other validated measures is one indication of the degree to which the CES is valid. The CES total survey score had significant correlations with several subscales of the SF-36, including BP, SF, GH, and MH. Although the SF-36 is a general measure and differs in scope from this and other outcomes measures used in otolaryngology,[11-14] these correlations are in the areas of the SF-36 where one would postulate convergence based on usual patient complaints. On the other hand, the lack of a significant difference in SF-36 scores between CSOM patients and the general population demonstrates that the SF-36 is not discriminating enough to be used as the only measure in CSOM and that an otitis media–specific outcomes measure such as the CES is needed.

Responsiveness, or sensitivity to longitudinal change, is the ability of a health instrument to detect change over time. The standardized responsiveness mean is one measure of this sensitivity. The responsiveness of .42 for the CES indicates moderate responsiveness. (A value of .2 or less is poor, .5 is moderate, and .8 or greater is excellent responsiveness.[15]) Hence, the CES is a useful instrument for clinical studies of intervention therapies for CSOM, including surgery and medical treatments.

There is a trade-off between precision and patient compliance. A lengthy questionnaire with more detailed items will increase discriminating power, but will inevitably increase both response and administrative burdens in a clinical setting. Patient compliance with these measures is a necessary part of ongoing outcomes management systems. A health measure must be short, easy to understand, and easy to administer without sacrificing its precision; the CES meets these criteria.

CONCLUSION

The CES is a new, disease-specific outcomes measure of CSOM. It provides a global score as well as subdomain scores for patient symptoms, activity restriction, and medical resource utilization. It is valid, reliable, and sensitive to clinical change. The CES is a significant and distinct advance in the ability to evaluate quality-of-life outcomes in CSOM.

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MICROSURGICAL ANATOMY OF THE PERIGENICULATE GANGLION AREA AS SEEN FROM A TRANSLABYRINTHINE APPROACH

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Detailed anatomic knowledge of the microsurgical anatomy of the perigeniculate ganglion area is essential to probing adjacent to the facial nerve by a translabyrinthine approach. This study was designed to investigate the surgical anatomy of the perigeniculate ganglion area of the facial nerve from a translabyrinthine point of view. We dissected 15 human temporal bones under a microscope, measured the lengths of the tympanic segment and the labyrinthine segment by a middle cranial fossa approach, and measured the angle between the tympanic and labyrinthine segments by a translabyrinthine approach. The distance of the facial nerve from the cochleariform process to the geniculate ganglion was 3.8 ± 0.7 mm. The length of the labyrinthine segment of the facial nerve was 4 ± 0.8 mm. The angle between the tympanic and labyrinthine segments from a translabyrinthine point of view was 26° ± 5°. Precise knowledge about the microsurgical anatomy of the perigeniculate ganglion area of the facial nerve from a translabyrinthine viewpoint is imperative for facial nerve decompression by a translabyrinthine approach.

KEY WORDS — facial nerve, surgical anatomy, translabyrinthine approach.

INTRODUCTION

The translabyrinthine approach is a useful method for decompression of the facial nerve in traumatic facial nerve palsies or for the removal of cerebellopontine angle tumors in patients with nonserviceable hearing. This technique is known as the most direct route for approaching the cerebellopontine angle. In performing this procedure, it is essential to thoroughly understand the microsurgical anatomy of the labyrinthine segment and the perigeniculate ganglion of the facial nerve. There have been many investigations into the anatomy of the facial nerve, but none into the microsurgical anatomy of the perigeniculate ganglion area of the facial nerve from a translabyrinthine viewpoint.[1-3] The purpose of this study was to measure the anatomic landmarks of the perigeniculate ganglion of the facial nerve in order to set out practical guidelines for total facial nerve decompression by a translabyrinthine approach.

MATERIALS AND METHODS

Fifteen adult human temporal bones were dissected by a translabyrinthine approach. The mastoid, tympanic, labyrinthine, and internal auditory canal segments of the facial nerve were dissected with an operating microscope. The lengths of the tympanic segment, the labyrinthine segment, and the internal auditory canal segment of the facial nerve were measured by a middle cranial fossa approach. The tympanic segment was measured from just above the cochleariform process to the geniculate ganglion, while the labyrinthine segment was measured from the geniculate ganglion to the lateral end of the internal auditory canal segment, which is the meatal foramen. The angle between the 2 segments was also measured. For measuring the angle between the tympanic and labyrinthine segments, the temporal bone was turned away 45° from the surgeon and the operating microscope was turned 25° from the horizontal plane (Fig 1). A photograph was taken from this view (Fig 2), and the angle was measured with calipers (accuracy 0.05 mm) and a protractor (error 0.5°). The median of 3 measurements was calculated. To prevent interpersonal error, 1 person did all the measurements.

RESULTS

The results of microdissection of the perigeniculate

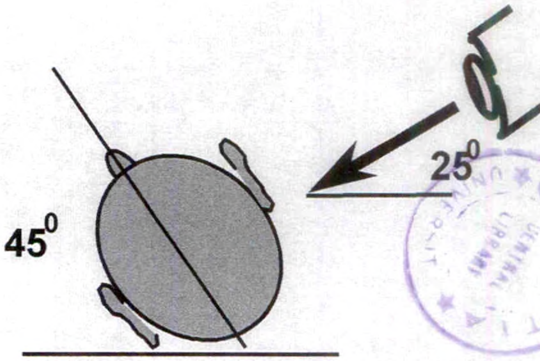

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Fig 1. Position of temporal bone measured. Patient is in supine position with head rotated 45° away from observer, and axis of microscope is raised 25° from horizontal plane.

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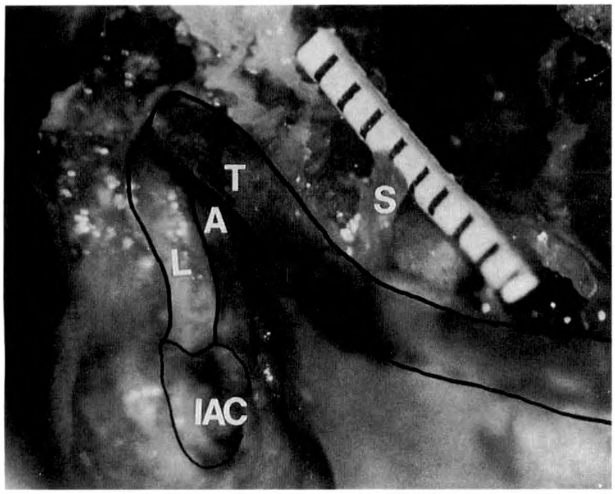

Fig 2. Exposed facial nerve (right side) in view of translabyrinthine approach. L — labyrinthine segment; T — tympanic segment; A — angle between labyrinthine and tympanic segments at geniculate ganglion; S — stapes; IAC — internal auditory canal.

ganglion of the facial nerve in adult temporal bones were as follows. The average lengths of the labyrinthine and tympanic (cochleariform process to geniculate ganglion) segments were 4 and 3.8 mm with standard deviations of 0.7 and 0.8 mm, respectively. The superior vestibular nerve, which was exposed just after labyrinthectomy, was a useful surgical landmark in finding the labyrinthine segment of the facial nerve. In other words, the labyrinthine segment of the facial nerve was easily found just anterior to Bill's bar after superior vestibular nerve exposure. The angle between the labyrinthine and tympanic segments from a translabyrinthine viewpoint was 26° with a standard deviation of 5°. The labyrinthine segment was cephalic to the tympanic segment.

DISCUSSION

Depending on the site of a facial nerve injury, facial nerve decompression can be performed by the transmastoid and middle cranial fossa approach in serviceable-hearing patients or by the translabyrinthine approach in nonserviceable-hearing patients.[4-6] The facial nerve can also be exposed in cases without facial nerve injury in order to remove skull base tumors by performing anterior or posterior facial nerve transposition.

Particularly, the translabyrinthine approach is a useful one in traumatic facial nerve palsies or in the removal of cerebellopontine angle tumors in nonserviceable-hearing patients. Thorough knowledge of microsurgical anatomy around the labyrinthine segment and geniculate ganglion is essential in performing this approach.[7,8]

The intratemporal facial nerve is composed of the mastoid, tympanic, labyrinthine, geniculate ganglion, and internal auditory canal segments. The labyrinthine segment is the thinnest segment, with a diameter of about 0.68 mm. To prevent traumatic injuries, careful dissection is necessary in surgeries around this segment.[3,7]

The tympanic segment of the facial nerve, which runs from the geniculate ganglion to the mastoid segment, is about 11 mm long.[9,10] Because the transitional part from the tympanic segment to the mastoid segment is broad and curved with a large radius, the cochleariform process may be a more constant surgical landmark in measuring the length of the tympanic segment. In this study, the average length of the tympanic segment from the geniculate ganglion to the cochleariform process was 3.8 mm. In a previous report about the anatomy of the anterior epitympanic space, the cochleariform process was within several millimeters of the geniculate ganglion, similar to our results.[11] The angle between the labyrinthine and tympanic segments is about 75° when one is looking perpendicular to the plane between these 2 segments; however, this angle can vary according to what kind of view the surgeon is looking at.[9,10,12] The authors measured this angle from the view that can be seen when performing a translabyrinthine approach (the patient lying supine with his or her head turned 45° from the surgeon and the operating microscope turned 25° from the horizontal plane). The average angle in this study was 26°. This angle can vary according to the position of the patient's head or of the operating microscope during the operation. (The angle increases when the patient's head is turned more than 45° away from the surgeon.) After mastoidectomy, the tympanic segment of the facial nerve was exposed. The length between the cochleariform process and the geniculate ganglion was 3.8 mm. The labyrinthine segment was acutely turned in the direction of the lateral end of the internal auditory canal at an average of 26°, and was cephalic to the tympanic segment. The average length of the labyrinthine segment from the geniculate ganglion to the lateral end of the internal auditory canal was 4 mm. This length looks shorter from a translabyrinthine point of view. In order to expose the internal auditory canal segment of the facial nerve, the medial wall of the vestibule, which is the lateral wall of the internal auditory canal, should be treated very carefully, because this is where the cochlear and vestibular nerves pass through to the inner ear. The ampulla of the superior semicircular canal is a useful landmark for the lateral end of the superior vestibular nerve, Bill's bar, and the facial nerve. In other words, the superior vestibular nerve can be found at the ampulla of the superior semicircular canal. Bill's bar is found just medial to the superior vestibular nerve, and the facial nerve just medial to Bill's bar. By means of the above-mentioned landmarks, we found the facial nerve could be easily identified. In order to prevent facial nerve injury of the external genu and tympanic segment during labyrinthectomy, the anterior wall of the lateral semicircular canal should be preserved. (However, in order to get a wider exposure in the removal of basal skull tumors, the facial nerve sometimes has to be transpositioned. In these cases, the anterior wall of the lateral semicircular canal should be totally removed.)

By means of the above-mentioned technique and surgical landmarks, the facial nerve can be completely exposed without injury. We found we could apply this technique in the decompression of traumatic facial nerve palsies and in the removal of skull base tumors.

In summary, the microsurgical landmarks of the perigeniculate ganglion from a translabyrinthine point of view were as follows. The length of the tympanic segment from the cochleariform process to the geniculate ganglion was 3.8 ± 0.7 mm. The length of the labyrinthine segment was 4 ± 0.8 mm. These segments look shorter from a translabyrinthine point of view. The angle between the labyrinthine and tympanic segments of the facial nerve from a translabyrinthine approach was 26° ± 5°.

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DISTRIBUTION AND LOCATION OF IMMUNOREACTIVE ATRIAL NATRIURETIC PEPTIDES IN MIDDLE EAR MUCOSA OF GUINEA PIG

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The distribution and location of atrial natriuretic peptides (ANP) in the middle ear mucosa (MEM) of guinea pigs were studied with immunocytochemistry (avidin-biotin-peroxidase complex method) and immuno–electron microscopy. A great quantity of ANP-immunoreactive (ANP-IR) cells, including oval small cells and irregular large cells, was observed in the tissue of the MEM. The strong ANP-IR product was observed in the inner membrane of the partial capillary walls. Under immuno–electron microscopy, the ANP-IR product was found to be spheres of 250 to 350 nm in diameter. The results of this study suggest that the MEM has the function of secreting ANP. This paper provides the morphological basis for the study of ANP's function in the MEM.

KEY WORDS — atrial natriuretic peptides, immunocytochemistry, middle ear mucosa.

INTRODUCTION

The tissue structure and function of the middle ear mucosa (MEM) have been studied by many authors.[1,2] It has been confirmed that the MEM has the function of secreting mucus and that it plays a role in the transport function of the ciliary system. There are 3 types of secreting cells, including light, dark, and mixed sphere cells, in the MEM epithelial layer.[3,4] In this experiment, atrial natriuretic peptide–immunoreactive (ANP-IR) spheres were confirmed for the first time in MEM tissue by immunocytochemistry and immuno–electron microscopy. This paper suggests that the ANP-IR spheres may be dark sphere cells, and discusses the function of ANP in the MEM.

MATERIALS AND METHODS

Ten white guinea pigs with an intact Preyer's reflex, weighing 300 to 350 g, were anesthetized with intraperitoneal pentobarbitone (30 mg/kg). Thoracotomy and aortic catheterization were performed. After washing out the blood with physiological saline, 700 mL 4% paraformaldehyde and 0.5% glutaraldehyde were perfused rapidly and then slowly for 1 hour. The temporal bone was removed, and the MEM was dissected under a dissection microscope. The MEM was left in 4% paraformaldehyde for 12 hours, then transferred into a 25% solution of sucrose phosphorylase and left for 4 to 6 hours. It was incubated in 0.5% methyl alcohol dihydric water, 0.5% Triton X-100, and sheep serum (1:70) for 30 minutes at 37°C, and mounted onto slides. A 1:600 first antibody (anti-ANP, Sigma) was dropped onto the slide samples and incubated for 48 to 72 hours at 4°C, then a sheep anti-rabbit IgG (1:200 vector) labeled with biotin and a complex of horseradish peroxidase (1:200 vector) labeled with avidin were incubated, both 30 minutes at 37°C. The specimens were re-

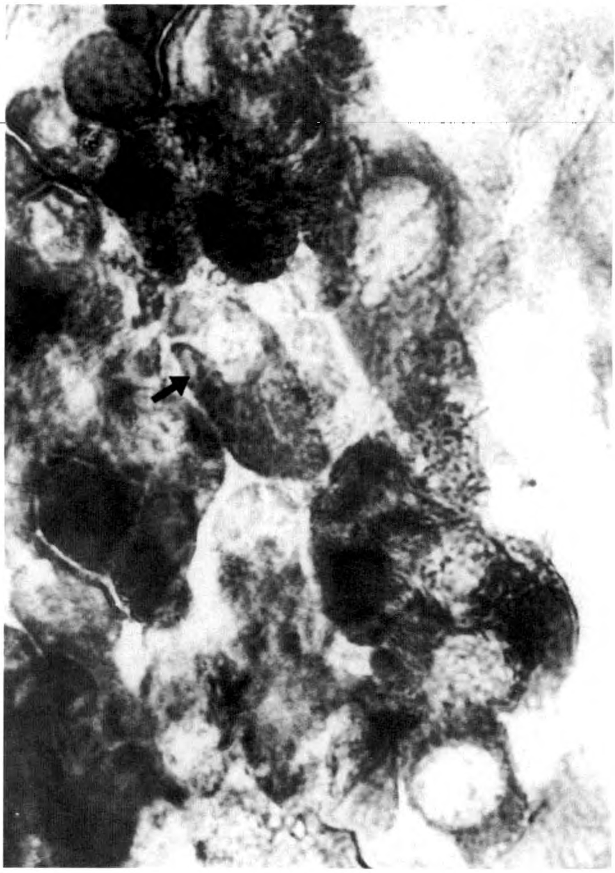

Fig 1. Middle ear mucosa (MEM) of promontorium tympanis, showing atrial natriuretic peptide–immunoreactive (ANP-IR) cells that are dense and small-volume and have nucleus located in lateral part (arrow; original ×1,320).

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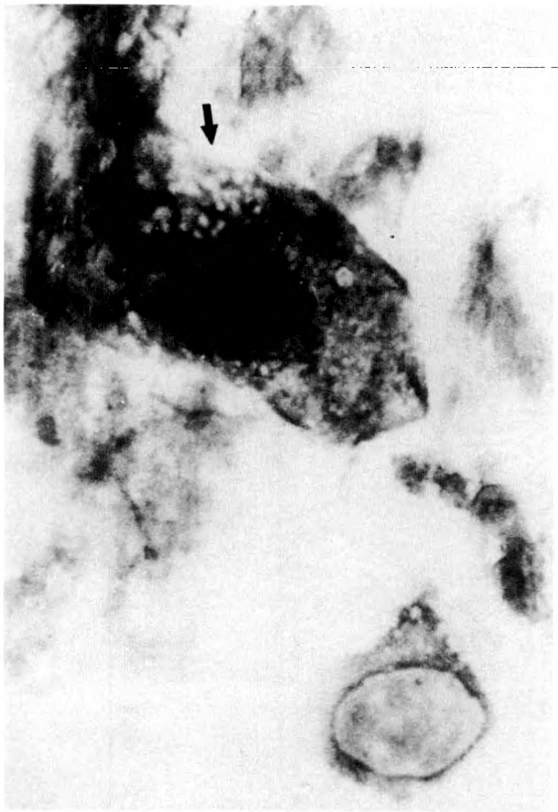

Fig 2. MEM's promontorium tympanis, showing cells that include mucus particles and ANP-IR particles (arrow; original ×1,320).

acted in diaminobenzidine dihydric water for 5 to 10 minutes, rinsed with 0.01-mol/L potassium phosphate, dried naturally at room temperature, dehydrated, mounted with gum, and photographed under a light microscope.

Other MEM specimens were frozen 3 times with liquid nitrogen. The procedures of preparation and immunoreaction adopted were similar to those described above, except that Triton X-100 was not used. After being stained, they were treated with osmic acid and uranium stain, dehydrated with gradient acetone, coated with epoxy propane and Epon 812, ultrathin-sectioned, stained for immuno–electron microscopic observation with a JEOL-2000EX transmission electron microscope, and photographed. For controls, rabbit serum was substituted for the antibody anti-ANP, and the treatment was otherwise the same.

RESULTS

Under a light microscope, the ANP-IR cells can be divided into 2 types: dense, small-volume cells with the nucleus located laterally (Fig 1), and large-volume cells that contain mucus particles and ANP-IR products within the cytoplasm. Both of these kinds of ANP-IR cells were found in the MEM of the promontorium of the guinea pig (Fig 2). The ANP-IR products were found in the vascular endangium of the MEM (Fig 3). Under transmission electron microscopy, densely distributed mucus particles of 300 to 600 nm in diameter were found in the cytoplasm of nonciliated secreted particle cells (Fig 4). Both mucus particles and ANP-IR particles of 250 to 350 nm in diameter were found in the cytoplasm of goblet cells (Fig 5). Many ANP-IR particles were seen in the cytoplasm of vascular endothelial cells (Fig 6).

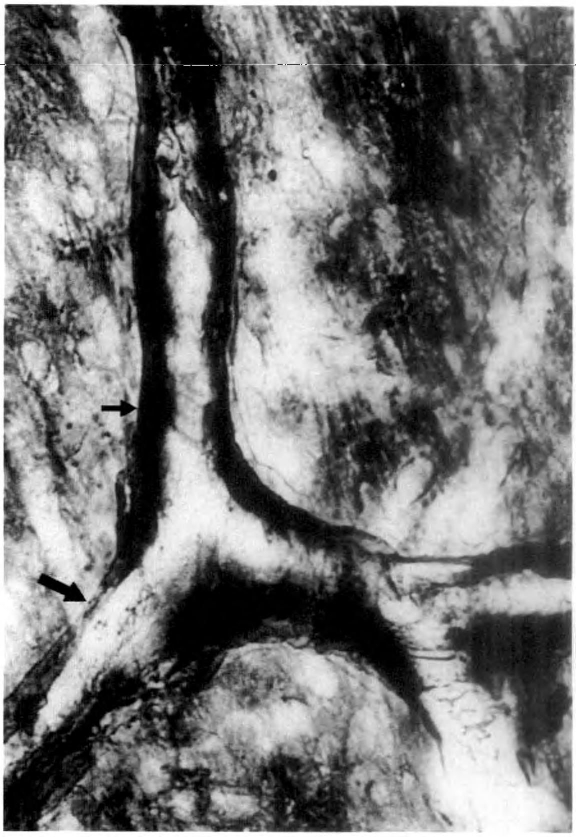

Fig 3. In MEM, strong ANP-IR product is seen in endangium (small arrow), but part of endangium (large arrow) is ANP-negative (original ×1,320).

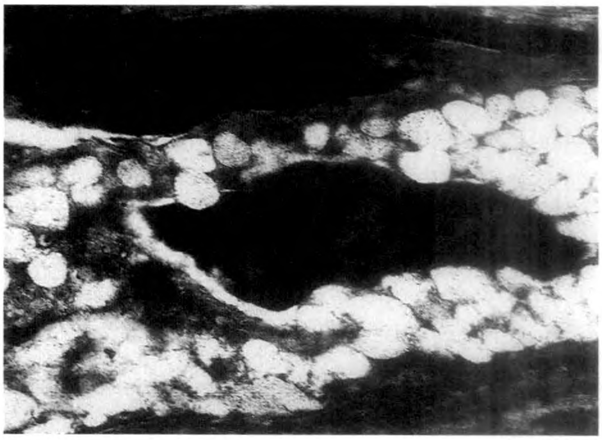

Fig 4. Many mucus particles of 300 to 600 nm in diameter, densely distributed, are found in cytoplasm of nonciliated secreted particle cells (original ×10,000).

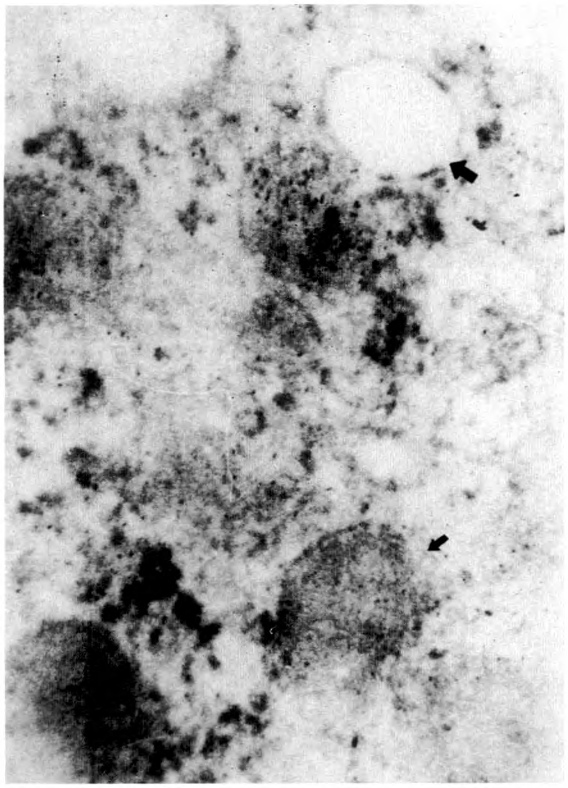

Fig 5. Both mucus (large arrow) and ANP-IR particles (small arrow) coexist in cytoplasm of goblet cells (original ×60,000).

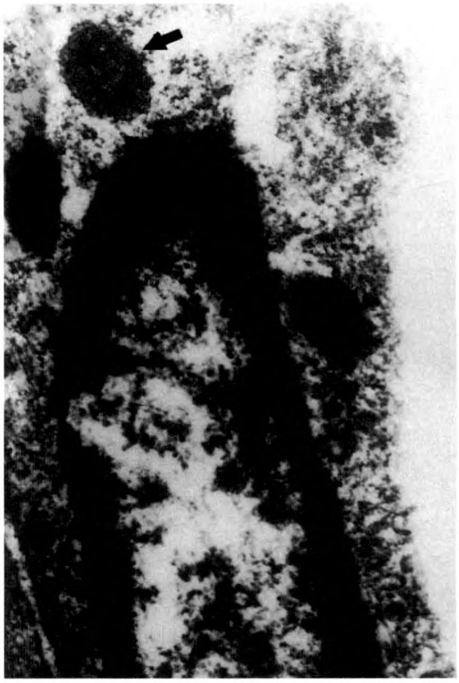

Fig 6. ANP-IR particles are markedly seen in cytoplasm of vascular endotheliocytes in MEM (arrow; original ×30,000).

DISCUSSION

In MEM, Sade[5] found mucous compositions in goblet cells, mucous gland cells, and mucus drops of common cells, and put forward that there exists a ciliated transport system in the middle ear of the human body. Mucus-secreting particle cells, including light, dark, and mixed-particle cells, were found by Maeda et al[3] and Albiin et al[4] in the MEM cells of humans and animals. They confirmed that the light particles are mucopolysaccharides, with periodic acid–Schiff staining, but they did not know the function of the dark particles of 250 to 350 nm in diameter, and guessed that it might be the synthesizing and secretion of enzyme and protein. In this experiment, mucus particle cells, ANP-IR particle cells, and mixed-particle cells were found in the MEM of guinea pigs by immunocytochemistry (avidin-biotin-immunoperoxidase method) and immuno–electron microscopy. The way that dark particles are excreted is the same as the way that ANP is released.[4,6] I consider the dark particles to be ANP-IR spheres.

In general, researchers think that the MEM is isogenous with respiratory epithelium. Atrial natriuretic peptide–immunoreactive product has been found in nasal mucosa, trachea, and lung. In pneumonedema, it has been confirmed that the level of ANP, and its connective power with its receptor, are reduced. The ANP in the lung vasculature, and its impairment, may have an important role in the process of pneumonedema occurrence.[7] The effect of ANP in the MEM and in the occurrence of otitis media needs further study.

In endothelial cells of the aorta and heart vasculature, ANP may dilate the arteries and increase the organ's blood flow.[8] I found ANP-IR products in endothelial cells of the MEM vasculature in normal guinea pigs, and this finding prompts me to suggest that ANP has an important role in regulation of the MEM's blood circulation.

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EFFECT OF PENTOXIFYLLINE ON THE HEALING OF GUINEA PIG TYMPANIC MEMBRANE

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Although most tympanic membrane perforations heal spontaneously, persistent perforations frequently require treatment by otolaryngologists. Initial management strategies include keeping the ear dry, ensuring aural hygiene, and using topical antibiotics. For persistent perforations, paper patching or myringoplasty may be required. Recently, agents such as hyaluronic acid and epidermal growth factor have been used to promote tympanic membrane healing. Similarly, pentoxifylline, a pharmaceutical agent with hemorrheological and antithrombotic properties, has been shown to increase perfusion and accelerate wound healing. This double-blinded prospective study attempts to examine the effect of pentoxifylline on tympanic membrane healing of 50 guinea pigs subjected to myringotomy. Serial examinations and histopathologic sectioning of the tympanic membranes revealed no significant difference in rate of healing or quality of repair between the pentoxifylline and control groups.

KEY WORDS — guinea pig, pentoxifylline, tympanic membrane perforation.

INTRODUCTION

Tympanic membrane perforations result from various causes, and most heal spontaneously. Many tympanic membrane perforations are secondary to trauma, often caused by cotton-tipped swabs or open-hand blows to the ear.[1] A large proportion of tympanic membrane perforations are secondary to acute otitis media, although the incidence has decreased since the use of antibiotics.[1] Another important cause is chronic otitis media, which is characterized by tympanic membrane perforation and suppurative exudate. Finally, failure of the tympanic membrane to heal after the extrusion of ventilation tubes, which are placed in over 300,000 patients each year in North America, is another cause of persistent tympanic membrane perforation.[1] Symptoms of tympanic membrane perforation include mild conductive hearing loss, aural fullness, and mild tinnitus. Any symptoms of vertigo or severe sensorineural hearing loss could indicate damage to the inner ear. Any findings of conductive hearing loss greater than 30 dB could mean ossicular chain disruption.

Treatment of tympanic membrane perforations arising from trauma or acute otitis should begin with conservative measures such as keeping the affected ear dry, ensuring adequate aural hygiene, and treatment with antibiotic-steroid drops if infection or inflammation is present. About 90% of traumatic perforations heal spontaneously, especially if less than 25% of the tympanic membrane surface is involved.[2] Factors that reduce the likelihood of spontaneous healing are large size, infection, and foreign bodies.

Paper patching, although advocated by some clinicians, has been shown to have no effect on the rate or outcome of healing.[3] However, in patients with tympanic membrane perforations who complain of mild hearing loss, tinnitus, or aural fullness, paper patching is helpful during the healing period. Another alternative is covering the defect with 1% hyaluronic acid, a highly viscous glycosaminoglycan that has been shown to speed the closure of traumatic perforations.[4-9] Similarly, epidermal growth factor has been shown to promote tympanic membrane healing in animals.[10,11] These agents may provide an effective alternative to myringoplasty in the treatment of persistent tympanic membrane perforations.

Pentoxifylline (Trental), a methylxanthine possess-

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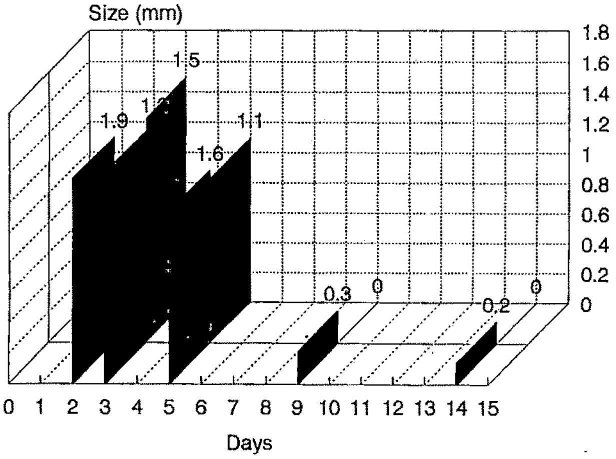

Fig 1. Average size of tympanic membrane perforations in pentoxifylline (darker bars) and control (lighter bars) groups during postoperative period.

ing hemorrheological and antithrombotic properties, has been demonstrated to improve perfusion and wound healing in a variety of pathological conditions. Pentoxifylline is commonly used to treat patients with peripheral vascular disease. Pentoxifylline has clearly proven effective in improving blood flow, tissue oxygenation, and ulcer healing in these patients.[12-17] Pentoxifylline has also been used to shorten the healing time of femoral and tibial diaphyseal fractures and duodenal ulcers.[18,19] A study indicates that pentoxifylline, administered with several other agents that influence coagulation, shortens the treatment period of burn patients by causing early eschar separation and wound readiness for grafting.[20] Numerous studies also show that pentoxifylline significantly increases the survival of skin and myocutaneous flaps in animals.[21-25] Despite its ability to accelerate wound healing in a variety of conditions, pentoxifylline's effect on the healing of tympanic membrane damaged by trauma has never been studied. The goal of this investigation is to study the effect of pentoxifylline on the healing rate of tympanic membranes damaged by myringotomy.

METHODS AND MATERIALS

Under the approval of the Laboratory Animal Care and Use Committee of Naval Medical Center, Portsmouth, Virginia, and in accordance with the Animal Welfare Act, 50 adult male albino guinea pigs weighing 600 to 700 g were anesthetized with ketamine hydrochloride 40 mg/kg and xylazine hydrochloride 5 mg/kg administered via intraperitoneal injection and maintained by redosing with the same mixture every 30 minutes as needed for the duration of the experiment. With a myringotomy knife, standard 1 × 2-mm perforations were made anterior to the malleus in 98 tympanic membranes, and posterior to the malleus in 2 tympanic membranes. After the location and size of each perforation was recorded, 25 guinea pigs received 20 mg/kg of pentoxifylline, and 25 received saline by intraperitoneal injection every 12 hours for the next 14 days. Each animal in the 2 groups was labeled with a random number by the veterinary technicians, and the investigators were blinded to which animals received pentoxifylline and which received saline solution. The investigators remained blinded until the completion of the study. Daily otomicroscopic examinations of each guinea pig were conducted by the investigators in order to assess the healing of the tympanic membrane perforation. Five guinea pigs from each group were randomly sacrificed in a painless manner by means of pentobarbital (80 mg/kg by intraperitoneal injection) given 24 hours postmyringotomy, then 5 more each were sacrificed on days 3, 5, 9, and 14 in order to histologically characterize the tympanic membrane at different stages of repair.

After euthanasia, the external ear canals were divided and the temporal bones removed. The bullae were then opened and the temporal bones immersed in a buffered formalin. Next, the specimens were decalcified in formic acid and the tympanic membranes were trimmed, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The histologic specimens from each tympanic membrane were then evaluated with respect to the presence and size of persistent perforations, as well as the presence of inflammation, epithelial migration, and neovascularization during the course of healing.

Data were analyzed by the analysis of variance (ANOVA) method to determine whether the administration of pentoxifylline significantly influenced the rate of healing or the presence of epithelial migration, inflammation, or neovascularization.

RESULTS

No significant differences were observed between the 2 groups in the number of healed perforations ($p = .680$) or the size of residual perforations after 14 days ($p = .075$; Fig 1). In the pentoxifylline group, 80% of the perforations were completely closed at 14 days. The average size of the remaining perforations in the pentoxifylline group after 14 days was 0.2 mm. In the control group, 100% of the perforations were completely closed at 14 days.

Further, there were no significant differences in the presence of inflammation ($p = .732$), epithelial migration ($p = .481$), or neovascularization ($p = .503$) between the pentoxifylline and control groups. Both groups displayed inflammation and epithelial migration 3 days after myringotomy (Fig 2A). Capillary

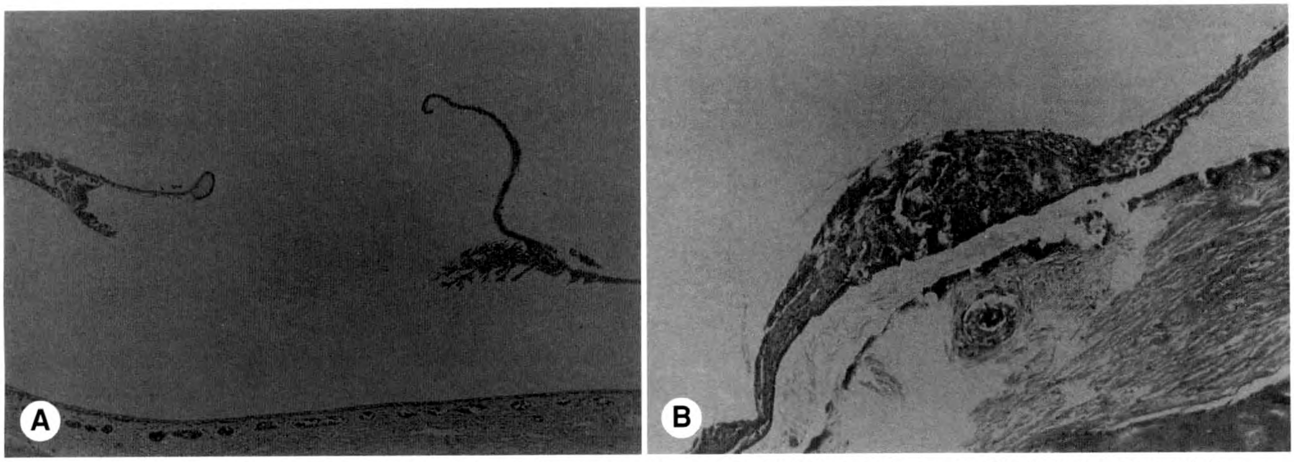

Fig 2. Tympanic membranes treated with pentoxifylline postmyringotomy. A) Inflammation and epithelial migration at 3 days. B) Neovascularization and fibrous reaction at 9 days.

proliferation was not observed in either group until after 5 to 9 days (Fig 2B). After day 3, a U-shaped ridge of epithelium was visible around the inferior edge of the perforations in both groups. In tympanic membranes with completely closed perforations, the epithelial layers were intact, whereas the middle fibrous layer remained absent in the wound.

DISCUSSION

The guinea pig is one of the most accepted animal models for the study of tympanic membrane healing. The guinea pig has proven to be an excellent model for the study of surface migration of tympanic epithelium and the healing of tympanic membrane perforations.[26-30] The gross and microscopic anatomy of the guinea pig tympanic membrane is similar to that of the human tympanic membrane. The outer epidermal layer of the guinea pig tympanic membrane consists of 2 to 5 layers of thin nucleated cells covered by 1 to 4 layers of stratum corneum. A fibrous middle layer, composed of a radial layer and a circumferential layer, separates the epidermis from the single layer of middle ear mucosa.[31] The migration of tympanic epithelium in the guinea pig occurs in an inferior-to-superior direction at a rate of 0.5 to 1 mm per day, a rate 10 times faster than that in humans.[32] Thus, healing of the guinea pig tympanic membrane occurs rapidly and is easily observed in a short period of time.

Various authors have noted that the initial response to trauma in the guinea pig tympanic membrane is hypertrophy and hyperplasia of the epithelial cells near the wound edge.[28-30] This is followed by movement of the stratum corneum across the perforation. Because the stratum corneum is essentially dead tissue, it is carried superiorly by migration of the epidermis on both sides of the perforation. Investigators have observed the formation of a U-shaped ridge of epidermis heaped around the inferior margin of the perforation and extending superiorly. This U-shaped ridge is eventually pulled over the perforation.[28-30] It is significant that the epidermis covers the defect in the tympanic membrane without the support of an underlying stroma, thus forming the matrix on which deeper connective tissue cells can migrate to complete the healing process.

The epidermal reaction of the guinea pig tympanic membrane seems to begin about 24 hours after trauma. There is a delay of about 2 days between the epidermal reaction and the onset of the vascular and fibrous reaction, which consists of small congested capillaries intermingled with plump fibroblasts and fibrous tissue. Fibrous tissue does not fill the perforation until 9 days after trauma.[30] Several authors reported that the mucosal layer of the tympanic membrane remains relatively inactive and plays only a minor role in healing.[28,30] Furthermore, epidermal cells of the guinea pig tympanic membrane undergo distinct shape changes and alterations of F-actin microfilaments during the healing process.[28] Over time, these changes are seen far from the wound, but without inflammation or hyperplasia. The mucosal cells, on the other hand, do not undergo shape changes or F-actin reorganization. Because of this extensive experience with the guinea pig, it is an excellent model for studying the effect of pentoxifylline on tympanic membrane healing.

In this study, our goal was to determine the effects of pentoxifylline on tympanic membrane healing after myringotomy. Pentoxifylline has been shown to increase the deformability of erythrocytes, thus leading to improved blood flow and tissue oxygenation. Pentoxifylline has also been found to have a similar effect on white blood cells, specifically on monocytes and polymorphonuclear leukocytes.[33] By improving the ability of red and white blood cells to reach the site of injury, pentoxifylline has proven useful in promoting wound healing in many different tissues. Specifically, pentoxifylline has been shown to improve skin flap survival in rats when administered at a dose of 12.5 to 20 mg/kg by intraperitoneal injection twice a day.[21-23] However, pentoxifylline did not significantly affect the speed of tympanic membrane healing, nor was the normal sequence of healing in the tympanic membrane altered in this study. This result may be explained by examining the differences between tympanic membrane healing and healing in other tissues.

Injured tissue undergoes 5 well-recognized stages of healing: hemostasis, inflammation, proliferation, remodeling, and contraction. Although healing in the tympanic membrane also involves early hemostasis and inflammation, its proliferative and migratory phases of healing are unique. In most wounds, a granulation tissue bed forms initially and serves as the platform for epithelial migration. In the tympanic membrane, however, the epithelial layer first migrates over the wound, beginning on day 3. This is followed by capillary neogenesis and re-formation of the middle fibrous layer during days 5 to 9. Our observations also support this sequence of healing in the guinea pig tympanic membrane. Other studies have shown that the remodeling and thinning of the fibrous layer subsequently occurs over the following 2 weeks. Pentoxifylline did not increase inflammation in the guinea pig tympanic membrane by promoting the migration of polymorphonuclear leukocytes, nor did pentoxifylline significantly influence the fibrous phase of healing by increasing microcirculation and tissue oxygenation to the injured area during the 2 weeks after myringotomy. It is possible that pentoxifylline may affect remodeling of the fibrous middle layer by improving microcirculation and tissue oxygenation during the third or fourth week after injury, but this was beyond the scope of this study. It is clear that the tympanic membrane is unique because, unlike other tissues, it heals primarily by epithelial migration. Pentoxifylline was not observed to have a significant effect on epithelial migration and thus did not improve healing in the guinea pig tympanic membrane. Further, the expected influence on inflammation and fibrous reaction was not observed.

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OVEREXPRESSION OF FAS-LIGAND IN HUMAN NASAL POLYPS

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TAINAN, TAIWAN

Apoptosis mediated through the Fas/Fas-L system is essential in regulating immune function, developing organs, and conferring immune privilege. To illustrate the role of the Fas/Fas-L system in the pathogenesis of human nasal polyps, we investigated the transcripts and protein level of the Fas-L gene in 8 human nasal polyp tissues and 7 nasal turbinate mucosa specimens using reverse transcription–polymerase chain reaction and Western blotting. Localization of Fas-L was performed with immunohistochemistry. The transcripts of the Fas-L gene were detected at similar levels in both polyps and nasal mucosa. There was a significant overexpression of Fas-L protein on nasal polyps compared to nasal mucosa. Fas-L–positive cells were localized on the epithelial layers of cystically dilated glands and the down-growing epithelium of nasal polyps. Fas-L may play an important role in the pathogenesis of human nasal polyps, including cystic degeneration of submucosal glands and conferring of immune privilege to nasal polyp formation.

KEY WORDS — Fas, Fas-ligand, nasal polyp.

INTRODUCTION

Nasal polyps, though not a fatal disease in humans, is a common nasal disease with a high rate of recurrence. The pathogenesis of nasal polyps is still an enigma. Several findings indicate that infiltration of eosinophil cells,[1,2] mast cells,[3] or T- and B-lymphocytes[4] in the tissue of nasal polyps might have an influence on or a relationship to polyp formation. It is believed that etiologic factors may include pathogen infection, allergy, and immunologic disorders. On the basis of pathological findings, Tos and Mogensen[5] have proposed the "epithelium rupture theory" or "new gland formation theory" for polyp formation. They suggest that formation of the tubular gland from the epithelium may occur by down-growth of the basal cells into the lamina propria during the early stage of polyp formation. Questions regarding how those down-growing cells form the gland structure, with cystic degeneration following, and how they can evade immune surveillance remain unexplained.

Apoptosis mediated through the interaction of Fas and Fas-ligand (Fas-L) is now recognized as an important mechanism, not only in functional organ development, but also in pathological changes, such as cancer and autoimmune diseases.[6,7] Fas is expressed on a variety of cell types, including lymphocytes[8] and neutrophils,[9] whereas Fas-L is expressed predominantly on activated T cells,[10] spleen, and thymus. Expression of Fas-L in nonlymphoid tissues, such as eye, testis, and brain, may confer immune privilege to those tissues.[11,12] In order to illustrate the possible role of the Fas–Fas-L system in the pathogenesis of human nasal polyps, we investigated the expression of Fas-L in polyps.

MATERIALS AND METHODS

Tissues. Nasal polyps were obtained from 8 patients undergoing polypectomy under local anesthesia at the National Cheng Kung University Hospital, Tainan, Taiwan. A "nasal mucosa" control group was included, consisting of 7 patients undergoing inferior turbinectomy. These specimens were immediately frozen in liquid nitrogen after excision and stored at –80°C until analysis.

Reverse Transcription–Polymerase Chain Reaction. Total RNA was prepared by the RNeasy Total RNA kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). Total RNA was converted to complementary DNA (cDNA) using oligo-dT as a primer and StrataScript H-reverse transcriptase (Stragene). The reaction mixture was incubated at 37°C for 1 hour, and then heated to 90°C for 5 minutes to denature the reverse transcriptase. The generated cDNA was subjected to polymerase chain reaction (PCR) amplification on a DNA Thermal Cycler (Hybaid Omnigene, United Kingdom) as described previously by Yang et al.[13] The PCR products were fractionated by agarose electrophoresis, stained with ethidium bromide, and visualized under ultraviolet light.

Western Blotting. Tissues were minced on a frozen section machine. Cells were extracted with a buffer containing 1% Triton X-100, 10-mmol/L Tris hydrochloride, pH 7.4, 0.15-mol/L sodium chloride, 2 mg/mL aprotinin, and 50 µg/mL PMSF (phenyl-

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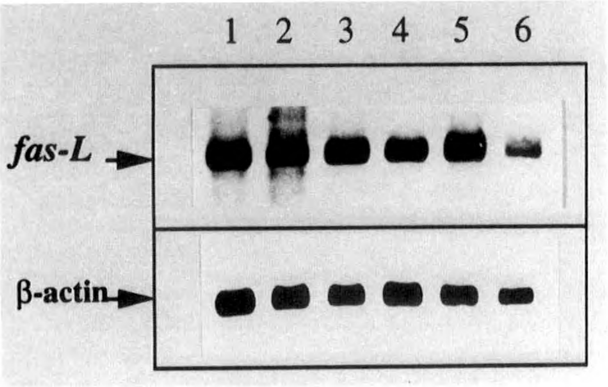

Fig 1. Reverse transcription–polymerase chain reaction (RT-PCR) analysis of Fas-ligand (Fas-L) gene. Total RNA of polyp tissues (lanes 1-3) and nasal turbinate mucosae (lanes 4-6) was isolated, and transcripts of Fas-L were analyzed by RT-PCR. β-Actin served as quantity control for RNA.

methylsulfonyl fluoride). Proteins were separated in a 12.5% sodium dodecyl sulfate–polyacrylamide gel and then electroblotted onto a nitrocellulose membrane. The proteins bound on the membrane were probed with rabbit IgG anti–Fas-L polyclonal antibody (N-20, Santa Cruz Biotechnology) at 4°C for 16 hours. Horseradish peroxidase–conjugated goat anti-mouse IgG antibody (Dako) was used as a secondary probe, and the immune complexes were made visible by fluorography with an enhanced chemiluminescence detection kit (Amersham International PLC, United Kingdom).

Immunohistochemistry. The localization of Fas-L was detected by immunostaining with an IgG anti–Fas-L polyclonal antibody. Cryosections of nasal samples were transferred onto glass slides coated with poly-L-lysine, fixed, heated with microwaves, and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). The fixed cells were permeabilized at room temperature for 15 minutes with 0.1% Triton-X-100 in PBS and were subsequently blocked by incubation with 0.5% bovine serum albumin. The cells were then hybridized with anti–Fas-L antibody for 3 hours followed by hybridization with a goat anti-rabbit IgG conjugated with horseradish peroxidase. After extensive washing with PBS, the cells were mounted on a glass slide over a drop of *p*-phenylenediamine–glycerol, examined by epifluorescence microscopy, and photographed.

RESULTS

Expression of Fas-L Gene. The transcripts of the Fas-L gene were detected by reverse transcription (RT)–PCR at a similar level in all nasal tissues analyzed, including 8 polyps and 7 specimens of nasal turbinate mucosa (Fig 1). By contrast, no transcripts of the Fas gene could be detected in the nasal mucosae or polyps analyzed. The expression of the Fas-L gene was further confirmed at the protein level by Western blotting. A significantly enhanced expression of the Fas-L protein was found for nasal polyps compared to nasal turbinate mucosae (Fig 2).

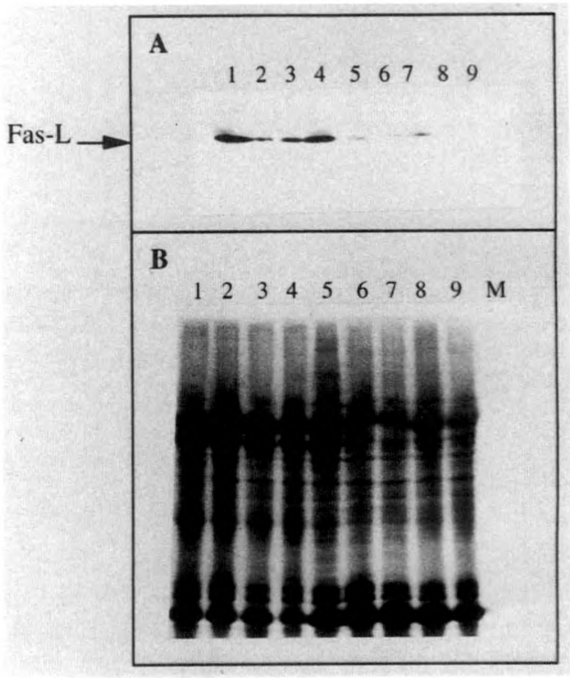

Fig 2. Western blot analysis of Fas-L. A) About 25 μg of total protein was separated on gel and probed with anti–Fas-L antibody. Lanes 1-4 — polyp tissues; lanes 5-9 — nasal turbinate mucosa. B) Silver staining of duplicate gel that served as control of total protein amount.

Localization of Fas-L Protein. Scattered Fas-L–positive cells were found in the nasal turbinate mucosae, while Fas-L–positive cells, strongly stained on the cell surface, were detected in the nasal polyps, which showed the characteristic morphology of epithelial cells. Two different locations of these Fas-L–positive cells were noted: one was in the down-growing epithelium of nasal polyps (Fig 3A), and the other was in the epithelial layer of cystically dilated glands (Fig 3B).

DISCUSSION

Fas-L expression was demonstrable in nasal polyps at both transcriptional and translational levels. Although transcripts of the Fas-L gene were detected in equal amounts in polyps and the inferior turbinates by semiquantitative RT-PCR, the former expressed a higher level of Fas-L protein. We had varied several PCR amplification conditions and did not see a significant difference at the transcriptional level. These results indicated that Fas-L expression was specifically enhanced in nasal polyps and might be regulated at the translational level.

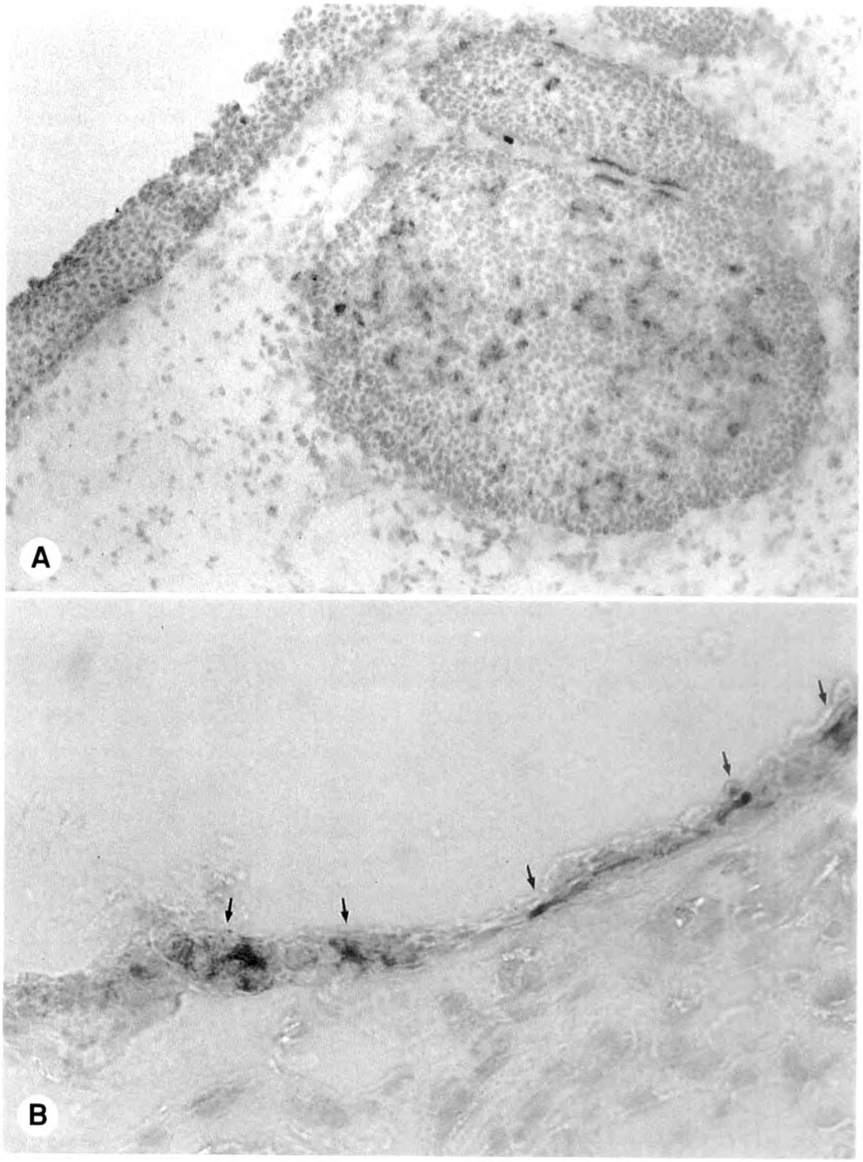

Fig 3. Immunohistochemical staining for Fas-L. A) Fas-L–positive cells are prominently noted on down-growing epithelium of human nasal polyp. B) Fas-L–positive staining (arrows) is found on epithelium of cystically dilated gland.

Several questions arise as to the functional significance of the mechanism of induction of Fas-L in nasal polyps. Nasal polyps are believed to be an inflammatory growth controlled by the local microenvironment.[14] For instance, viral infection is common in nasal mucosa, and triggers production of various colony-stimulating factors and interleukins by epithelial cells. Those factors may induce the expression of genes of the epithelium in an autocrine or paracrine manner.[15] It has been demonstrated in human immunodeficiency virus (HIV) infection that HIV may upregulate Fas-L in peripheral blood mononuclear cells.[16] It is likely that a local inflammatory factor or viral infection may stimulate the expression of Fas-L on epithelial cells.

Fas-L may contribute to the immune privilege of tumors such as hepatoma and melanoma.[17] These tumor cells not only escape Fas-mediated killing by down-regulation of the expression of Fas molecules, but also have the ability to actively kill T-lymphocytes by expressing Fas-L protein on the cell surface.[18-20] Whether Fas-L also confers the immune privilege of polyps awaits further study. On the other hand, apoptosis is also involved in polycystic kidney syndrome. Cystogenesis is accompanied by enhanced incorporation of 5-bromo-2'-deoxyuridine in cells within the cortex and medulla and transient apoptosis of cells within cysts and in the renal interstitium.[21] Fas-L was localized on the down-growing epithelium, which will form the new glands of the polyps afterward. In addition, intensive staining of Fas-L was noted in the epithelium of cystically dilated glands. We had tried but failed to show apoptotic cells by the TUNAL method[22] in well-formed dilated glands. Perhaps apoptosis occurs only at early stages of gland formation and cystogenesis. However, it is difficult to collect these samples just at that stage. If

Fas-L expression is a general strategy used by nasal polyps to escape immune rejection, pharmacological products that render infiltrating immunocompetent cells insensitive to Fas-L–induced killing will help to break the immunologic unresponsiveness of nasal polyps. In addition, inhibiting Fas-L production and consequently suppressing Fas-L cytotoxicity with drugs such as dexamethasone and all-trans retinoic acid[23] may be also beneficial and provide a complementary therapy for human nasal polyps.

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SECRETORY CELL DIFFERENTIATION AND MUCUS SECRETION IN CULTURES OF HUMAN NASAL EPITHELIAL CELLS: USE OF A MONOCLONAL ANTIBODY TO STUDY HUMAN NASAL MUCIN

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We have developed an air-liquid interface culture system for human nasal epithelial cells that differentiate into mucociliary phenotypes in a defined serum-free medium. Dissociated cells obtained from nasal polyps were cultured on a collagen gel substrate. At confluence, the cells lost characteristics of differentiated cells, and secretory cell and ciliated cell differentiation appeared after 7 days in an air-liquid interface. After 21 days, about half of the epithelial cells were stained with Alcian blue–periodic acid–Schiff stain or monoclonal antibody HCS18, which was directed against human nasal mucin specific for epithelial secretory (goblet) cells. The quantitative examination using the antibody HCS18 revealed that the antibody-reactive nasal mucin was secreted only on the apical side of the cultures, and interleukin-1β and tumor necrosis factor α stimulated these mucus secretions. The culture system with an antimucin monoclonal antibody developed in this study should be useful for studying polarized mucus secretion from human nasal epithelial cells.

KEY WORDS — cell culture, cell differentiation, ciliated cell, epithelial cell, goblet cell, human, interleukin-1β, monoclonal antibody, mucin, mucus secretion, nose, retinoic acid, secretory cell, tumor necrosis factor α.

INTRODUCTION

Mucus plays an important role in the defense mechanisms of the respiratory tract by trapping inhaled particles and microbial substances and allowing their removal by mucociliary clearance. Hypersecretion of mucus is an important characteristic of airway inflammation, such as rhinitis, sinusitis, tracheobronchitis, and bronchial asthma. Hypertrophic, hyperplastic, and metaplastic changes of secretory cells in the surface epithelium and submucosal glands are commonly observed in these affected epithelia in association with excess mucus. The mechanisms of mucus biosynthesis and secretion are not well understood, and it is desirable to establish an appropriate in vitro model to study secretory function in a conditioned environment.

Many types of explant or monolayer cell culture systems for various species have been reported for studying airway mucus secretion[1-3]; however, both systems have disadvantages. The explant culture contains other cell types: fibroblasts and inflammatory cells, along with the epithelial cells. It is difficult to study the direct effects on the epithelial cells, because these other cells may affect the epithelial cells' response. Monolayer cell culture consists of a pure population of epithelial cells, but the cells rapidly begin to lose their differentiated mucociliary characteristics when grown on a plastic surface. The physiological and biochemical functions of these cells would be different from those of airway epithelial cells in vivo.

In vitro cell culture systems for human nasal epithelial cells have been developed. Wu et al[4] reported the growth and differentiation of human nasal epithelial cells on a plastic plate, although ciliated cell or secretory cell differentiation was not induced. Hanamure et al[5] studied the ciliogenesis of human nasal epithelial cells by using a floating culture system. However, secretory cell differentiation was suppressed by ciliated cell differentiation in this model.

In the present study, to examine the mucus secretion from the airway epithelial cells, we developed an in vitro culture system for human nasal epithelial cells with many differentiated secretory cells by using an air-liquid interface culture using retinoic acid and a collagen gel substrate. The system used a serum-free medium, and its well-defined nature enabled us to study specific factors for mucus secretion of the epithelial secretory (goblet) cells. Already, we have developed 18 monoclonal antibodies against nasal mucin from patients with chronic sinusitis, and antibody HCS18 specifically reacted with nasal mucin of epithelial secretory (goblet) cells. Mucus secretion from the culture was quantitatively measured with

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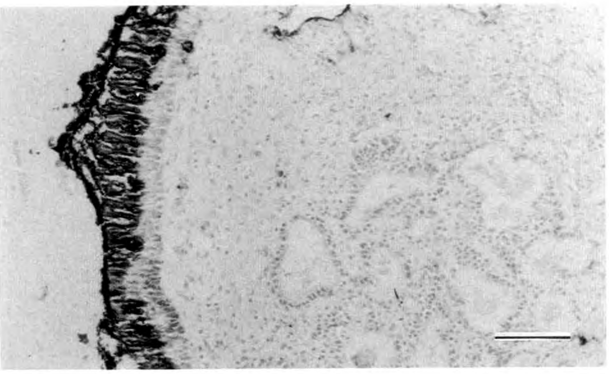

Fig 1. Immunohistochemistry of human nasal tissue with antimucin monoclonal antibody HCS18. This antibody stains all epithelial secretory (goblet) cells, but does not react with submucosal gland cells. Bar — 30 μm.

this novel monoclonal antibody.

MATERIALS AND METHODS

Cell Isolation. Human nasal epithelial cells were obtained from nasal polyps of patients with chronic sinusitis undergoing endonasal surgery. The stroma was removed and the epithelial cells were dissociated by incubation with a 1% solution of pronase (Sigma, St Louis, Mo) in Ham's F12 medium (Sigma) for 20 hours at 4°C. The cell suspensions were filtered through a 20-μm Nitex mesh, centrifuged at 500*g* for 10 minutes, washed, and resuspended in a serum-free hormone supplement culture medium.

Cell Culture. The culture medium was Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 supplemented with L-leucine (0.45 mmol/L), L-lysine (0.5 mmol/L), L-methionine (0.12 mmol/L), magnesium chloride (0.3 mmol/L), magnesium sulfate (0.4 mmol/L), calcium chloride (1.05 mmol/L), phenol red (8.6 mg/L), sodium bicarbonate (1.2 g/L), L-glutamine (6.5 mmol/L), penicillin-streptomycin (50 U/mL and 50 μg/mL), insulin (10 μg/mL), hydrocortisone (0.1 μg/mL), choleratoxin (0.1 μg/mL), transferrin (5 μg/mL), epidermal growth factor (25 ng/mL), endothelial cell growth supplement (7.5 μg/mL), bovine pituitary extract (50 μg/mL), HEPES (30 mmol/L), and bovine serum albumin (BSA, 3 mg/mL). All the reagents were purchased from Sigma except for penicillin-streptomycin (Gibco, Grand Island, NY).

Dissociated cells were plated onto Transwell-Col tissue culture inserts (24.5-mm diameter with 0.45-μm pore size, Coster, Cambridge, Mass), precoated with 0.4 mL of type I collagen gel (Cellmatrix, Nitta gelatin, Osaka, Japan), at a density of 5×10^4 cells per well in 0.5 mL of culture medium. The bottom compartment was filled with 2 mL of culture medium containing 10% fetal bovine serum (FBS, Cansera, Rexdale, Canada) for the first 24 hours of culture. The dishes were incubated at 37°C with 5% carbon dioxide in 95% air. After 24 hours, the medium in both compartments was replaced with culture medium containing 5×10^8 mol/L retinoic acid (Sigma). The medium was changed every other day, and an air-liquid interface was created when the cells became confluent at day 10 by removing the apical medium and filling only the bottom compartment with 2 mL of medium. After that point, the medium was changed every day because of the rapid acidification.

Cell Counting and Immunocytochemistry. Nasal epithelial cells grown on a collagen gel substrate were released with 0.5% trypsin–ethylenediaminetetraacetic acid at 37°C for 90 minutes. Cells were recovered by centrifugation and rinsed with phosphate-buffered saline (PBS), and duplicate samples of the cells were counted in a hemocytometer.

For immunocytochemistry, the dissociated cells were cytospinned at 500*g* for 3 minutes and fixed with acetone for 5 minutes. The slides were stained with Alcian blue–periodic acid–Schiff and hematoxylin (AB-PAS-H).

We have developed 18 monoclonal antibodies against purified human nasal mucin for studying secretory cell differentiation and mucus secretion. In the present study, monoclonal antibody HCS18 was selected for use because this antibody specifically reacts with secretory granules of the epithelial secretory (goblet) cells in the human nose (Fig 1).

The cytospinned slides were stained with this antibody. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide (H_2O_2)–methanol for 30 minutes. Nonspecific reactions were blocked by 1% BSA-PBS for 10 minutes at room temperature. The slides were incubated with hybridoma supernatant containing antimucin monoclonal antibody HCS18 diluted 1:5 in 1% BSA-PBS for 2 hours at room temperature. After washing with PBS, the slides were incubated with peroxidase-conjugated goat anti-mouse IgG and IgM antibody diluted 1:100 in 1% BSA-PBS for 1 hour at room temperature. Staining for peroxidase activity was done by a 5-minute incubation in diaminobenzidine tetrahydrochloride (DAB)–H_2O_2 solution (20 mg DAB and 10 μL 30% H_2O_2 in 100 mL of 0.05-mol/L Tris buffer, pH 7.6). The slides were counterstained with 1% methyl green.

Electron Microscopy. Cultures were fixed with 2.5% glutaraldehyde and were processed for transmission electron microscopy with osmium tetroxide postfixation, ethanol dehydration, and embedding in Epon. Ultrathin sections were obtained and stained with uranyl acetate and lead citrate. These were stud-

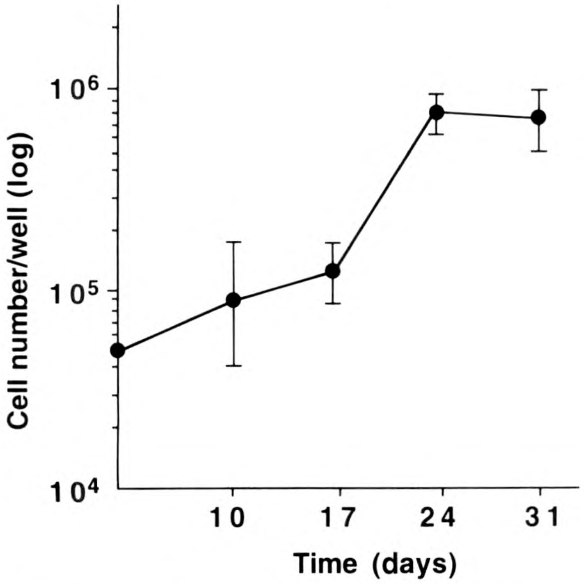

Fig 2. Growth of primary human nasal epithelial cells on Transwell-Col inserts with collagen gel substrate (n = 6).

ied in a JEM 100B electron microscope.

Slot Blot Analysis of Mucus Secretion Using Antimucin Monoclonal Antibody HCS18. The secreted mucus was collected by washing the apical surface of the culture with 0.5 mL of PBS; the medium of the bottom compartment was also collected. These samples (0.5 mL) were applied to nitrocellulose paper with a slot blot apparatus (Bio-Rad, Richmond, Calif). The nitrocellulose was stained by sequential incubation in 1) 3% gelatin in 0.02-mol/L Tris-buffered saline, pH 7.5 (TBS) for 30 minutes at room temperature, 2) antimucin monoclonal antibody HCS18 diluted 1:5 in 1% gelatin-TBS for 2 hours at room temperature, 3) peroxidase-conjugated goat anti-mouse IgG and IgM diluted 1:1,000 in 1% gelatin-TBS for 1 hour at room temperature, and 4) 20 mg DAB and 100 μL 3% H_2O_2 in 100 mL of 0.05-mol/L Tris buffer, pH 7.6.

Enzyme-Linked Immunosorbent Assay Using Antibody HCS18. Specimens and serial dilutions of "standard" purified nasal mucin in 0.05-mol/L sodium carbonate buffer (pH 9.3) were plated on 96-well tissue culture plates. The plates were incubated overnight at 4°C and washed 5 times with 0.01-mol/L PBS–1% Tween20. Then, the plates were incubated with purified monoclonal antibody HCS18 diluted 1:20,000 in 1% BSA-PBS for 1 hour at room temperature. After washing, the plates were incubated with peroxidase-conjugated goat anti-mouse IgG and IgM diluted 1:5,000 for 1 hour at room temperature. The plates were washed and developed by a 10-minute incubation in *o*-phenylenediamine (OPD) solution (20 mg OPD, 20 μL of 30% H_2O_2 in 200 mL of 0.2-mol/L sodium phosphate–0.1-mol/L citrate buffer, pH 5.0). The reaction was stopped by the addition of 50 μL of 4N sulfuric acid. Developed color in the plates was read at 490-nm wavelength in a microplate reader (ImmunoReader NJ-2000, InterMed, Osaka, Japan).

Statistics. All data are expressed as mean ± SD. Comparisons were performed with a Mann-Whitney test. Probability values of $p < .05$ were considered significant.

RESULTS

Growth of primary human nasal epithelial cells on Transwell-Col inserts with a collagen gel substrate was shown (Fig 2). The cells reached confluence in 7 to 10 days. At confluence, the cells showed a squamous and cobblestone appearance, and no ciliated cells were observed under the phase-contrast microscope. Transmission electron microscopy revealed 1 to 3 layers of flat or cuboidal epithelial cells with flattened nuclei (Fig 3). Microvilli were present on the apical cytoplasm, and the cells were connected by desmosomes. The cytoplasmic organelles were poorly developed, and the cells lacked any characteristics of mature epithelial cells, such as cilia and secretory granules.

The epithelial nature of these cells was confirmed by staining the cytospinned slides with the anticytokeratin monoclonal antibody AE1/AE3 (Progen Biotechnik, Heidelberg, Germany). All the epithelial cells in 10-day-old cultures were stained with AE1/AE3. The cells did not react with antivimentin monoclonal antibody (Biohit, Helsinki, Finland) or anticollagen type III monoclonal antibody (Sanbio, Uden, the Netherlands).

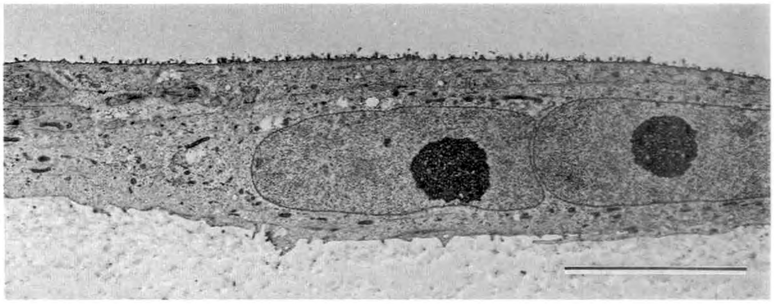

Fig 3. Transmission electron micrograph illustrating flat nasal epithelial cells of confluent culture at day 10. Cytoplasmic organelles are poorly developed, and cells have no characteristics of any mature cell types. Bar — 10 μm.

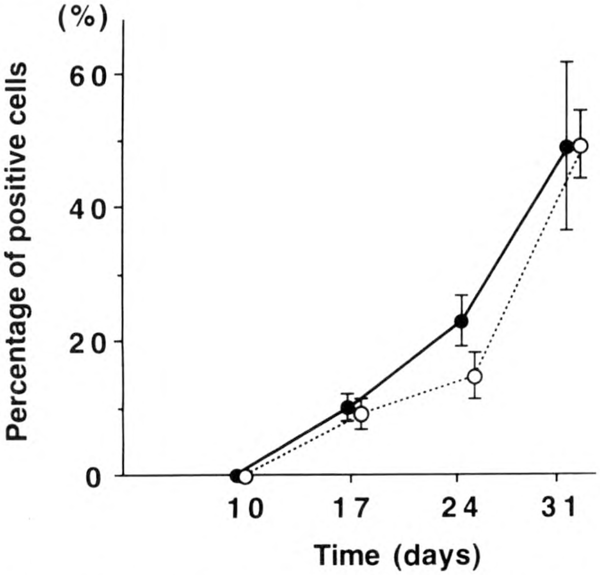

Fig 4. Percentage of Alcian blue–periodic acid–Schiff (AB-PAS)– or monoclonal antibody HCS18–positive epithelial cells of air-liquid interface cultures (n = 6). Percentage of positive cells was calculated by counting 300 cells in random fields on cytospinned slides at high-power magnification (×1,000). Open circles — AB-PAS; closed circles — HCS18.

At day 17, after 7 days in the air-liquid interface culture condition, a few ciliated cells, recognized by the beating of cilia, first appeared under phase-contrast microscopy. The scattered ciliated cells constituted less than 1% of all epithelial cells examined on the cytospinned slides. The number of ciliated cells did not increase after 17, 24, or 31 days in culture.

The degree of secretory cell differentiation was studied by staining the cytospinned slides with AB-PAS-H and monoclonal antibody HCS18. Figure 4 shows the percentages of AB-PAS– and HCS18-positive secretory cells in all epithelial cells. At day 10, no epithelial cells reacted with AB-PAS or HCS18. At day 17, after 7 days in the air-liquid interface culture condition, AB-PAS– and HCS18-positive secretory cells appeared. The number of these secretory cells increased after that day, and about half of the epithelial cells in culture were AB-PAS– or HCS18-positive secretory cells at day 31. Ultrastructurally, electron-lucent secretory granules were observed at the apical surface of these secretory cells, which contained endoplasmic reticulum and Golgi apparatus (Fig 5).

Slot blot analysis of the secreted mucus revealed that human nasal mucin reacted with monoclonal antibody HCS18 and was secreted from day 17 on, coincident with the first appearance of HCS18-positive secretory cells in the culture (Fig 6). The amount of HCS18-reactive mucin increased continuously after that day. The medium in the lower compartment of the culture did not react with HCS18. Only the samples collected from the apical side contained HCS18-reactive mucin, indicating that there was a polarity in mucus secretion from the cultured epithelial cells.

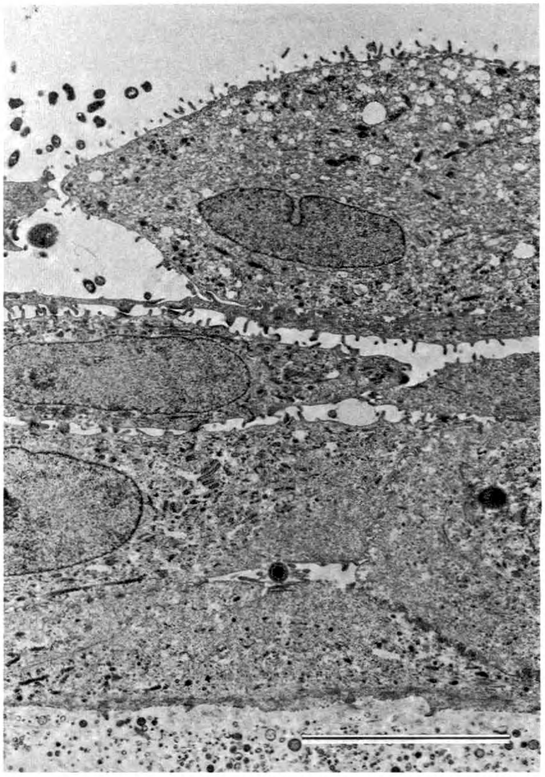

Fig 5. Transmission electron micrograph illustrating human nasal epithelial cells of air-liquid interface culture at day 31. Secretory cell with electron-lucent granules is shown at apical surface of culture. Bar — 10 μm.

The effects of interleukin (IL)–1β (Genzyme, Cambridge, Mass) and tumor necrosis factor α (TNF-α; Sigma) on mucus secretion from the cultured epithelial cells were examined at day 24, after 14 days in the air-liquid interface culture condition. The basolateral medium was exposed to IL-1β (20 ng/mL) or TNF-α (20 ng/mL) for 24 hours. The mucus secretion was quantitatively measured by enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody HCS18. Interleukin-1β and TNF-α significantly stimulated mucus secretion after a 24-hour incubation, and increased secretion was still observed at 24 hours and 48 hours after removal of the drugs (Fig 7).

DISCUSSION

We have developed an air-liquid interface culture system for secretory cell differentiation of human nasal epithelial cells in vitro. Recently, to obtain the differentiated phenotypes in primary cell cultures of respiratory epithelial cells, air-liquid interface cultures were used for tracheobronchial epithelial cells

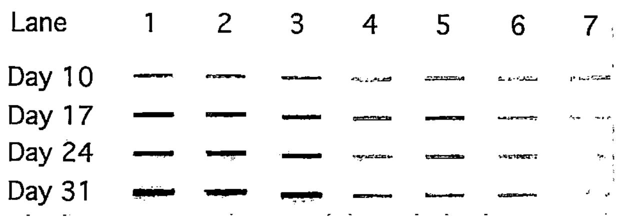

Fig 6. Slot blot analysis of secreted mucus obtained from apical side of air-liquid interface cultures. Lanes 1, 2, and 3 — samples collected from apical side by washing surface of cultures with 0.5 mL of phosphate-buffered saline; lanes 4, 5, and 6 — medium of bottom compartment; lane 7 — control culture medium. Nitrocellulose paper was stained with antimucin monoclonal antibody HCS18 as described in Materials and Methods.

of various species.[6-9] This is the first study to develop this culture system for human nasal epithelial cells. In studying the physiological and biochemical functions of airway epithelial cells, the development of an in vitro culture system for human nasal epithelial cells with differentiated phenotypes will be more useful than that for tracheobronchial cells, because a large amount of nasal tissue is easily obtained from patients undergoing endonasal surgery, and nasal epithelium shows inflammatory responses similar to those of the lower respiratory airways.

In the present study, human nasal epithelial cells were dissociated from freshly excised nasal polyps. A serum-free hormone supplement medium was used to study factors specific for secretory cell differentiation and mucus secretion. We added FBS (10%) during the first 24 hours of culture, because it facilitated the abundant cell attachment.[9] Shortly after the cells attached, the nasal epithelial cells lost the characteristics of differentiated phenotypes, and at confluence, the cultured epithelium was composed of poorly differentiated epithelial cells. After 7 days in the air-liquid interface culture condition, secretory cells and ciliated cells reappeared, and redifferentiation of the epithelial cells was observed. This cell culture system will be useful not only in studying physiological and biochemical functions of differentiated epithelial cells, but also in studying factors regulating epithelial cell differentiation.

The combination of retinoic acid, a collagen gel substratum, and an air-liquid interface at the level of the cell layer was a crucial factor for achieving secretory cell and ciliated cell differentiation.[9,10] The importance of retinoids as mucociliary differentiation agents for airway epithelial cells has been reported in various species.[11,12] The cultured cells in retinoid-depleted medium differentiated into a stratified squamous epithelium.[13,14] The collagen gel substratum and other extracellular matrices are important factors in the attachment, growth, and differentiation of cultured airway epithelial cells. The cultured cells are usually poorly differentiated, but when the cells were grown on type I collagen gel, a columnar mucus-secreting epithelium was established.[9,15] Davenport and Nettesheim[10] have reported that the type I collagen gel substratum was critical for ciliated cell differentiation in air-liquid interface cultures, and that it accelerated the onset of secretory cell differentiation.

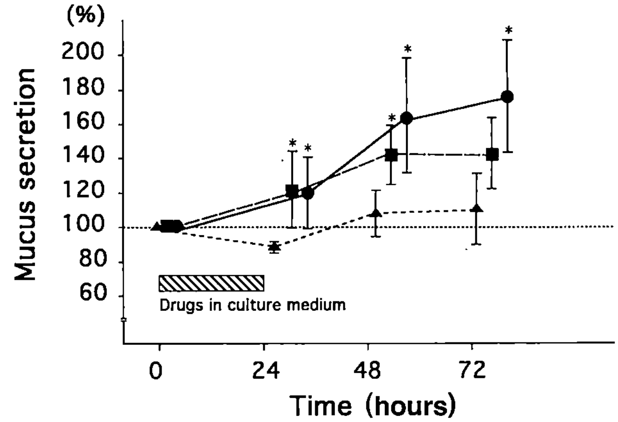

Fig 7. Effects of interleukin (IL)–1β (20 ng/mL) and tumor necrosis factor α (TNF-α; 20 ng/mL) on mucus secretion from cultured epithelial cells ($n = 4$). Drugs were added to basolateral medium and incubated for 24 hours at day 24, after 14 days in air-liquid interface condition. Mucus secretion was quantitatively examined by enzyme-linked immunosorbent assay with antimucin monoclonal antibody HCS18 as described in Materials and Methods. Circles — IL-1β; squares — TNF-α; triangles — control medium; asterisks — $p < .05$, versus control medium.

Increased mucociliary differentiation of the tracheal epithelial cells has been reported in air-liquid interface cultures compared with immersed cultures.[9,16] In interface cultures, the nutrient supply was reached only from the basolateral side of the epithelium, and the apical surface was exposed to air. That was similar to in vivo environments, and morphological polarity of the differentiated epithelium was established. In the present study, mucus secretion was directed to the apical surface. That none was found in the basolateral medium indicates that this culture system retains the polarized functions of mucus secretion as well as morphological polarity. The air-liquid interface culture enables more detailed studies of epithelial cell functions, because the stimulation or response of the cells can be examined from the apical and basolateral sides of the epithelium separately.

In the present study, secretory cell differentiation was examined with AB-PAS staining and immunocytochemical staining with antimucin monoclonal antibody HCS18. The percentages of AB-PAS–positive and HCS18-positive cells were similar at each time point. After 3 weeks in the air-liquid interface, about half of the cultured cells were secretory cells that reacted with AB-PAS and HCS18. These results indicate that most of the secretory cells detected by AB-PAS also reacted with HCS18 monoclonal antibody. The antibody HCS18 was developed against purified human nasal mucin obtained from patients with chronic sinusitis. The antibody is the IgG1 form and reacted with high–molecular weight mucous glycoprotein in a Western blot analysis (data not shown). Immunohistochemical examination showed that the antibody specifically reacted with epithelial secretory (goblet) cells in human nasal epithelium, and did not stain submucosal gland cells. These unique characteristics of the antibody revealed that most of the differentiated secretory cells in the cultures synthesized the epithelial cell–specific mucin of human nasal epithelium.

Because airway secretions are complex mixtures of proteins, an antimucin monoclonal antibody will be a useful tool for examining secreted mucus. Monoclonal antibodies developed against tracheal mucin have been reported and have been used for studying mucus secretion from tracheobronchial cells.[17,18] These antibodies were of no use in studying nasal mucin, because of the different staining properties observed between nasal and tracheal secretory cells. We have produced the first monoclonal antibodies against human nasal mucin for studying mucus secretion from nasal epithelial cells. In the present study, increased mucus secretion was measured with the antibody HCS18 and the ELISA method developed for quantitative examinations.

Interleukin-1β and TNF-α are involved in various inflammatory responses as inflammatory cytokines. Macrophages, monocytes, and a variety of cell types produce these cytokines in response to bacterial infections[19] and allergic inflammations.[20] It has been reported that TNF-α stimulates an increased secretion of mucin from cultured tracheal epithelial cells of guinea pigs[21]; however, the effects of IL-1β on epithelial mucus secretion are unknown. The present study revealed that both IL-1β and TNF-α stimulated mucus secretion from cultured nasal epithelial cells and that this increased secretion continued for 48 hours after removal of these cytokines.

We have presented an air-liquid interface culture system in a serum-free medium for human nasal epithelial cells with many differentiated secretory cells. Polarized mucus secretion was observed with antimucin monoclonal antibody HCS18, developed against human nasal mucin. This cell culture system will be useful for studying factors regulating mucus secretion from the airway epithelial cells.

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EUROPEAN ACADEMY OF FACIAL PLASTIC SURGERY

The annual meeting of the European Academy of Facial Plastic Surgery will be held September 15-17, 2000, in Thessaloniki, Greece. For information, contact Prof Dr J. Daniilidis, ENT Dept, Aristotelian University of Thessaloniki-AHEPA Hospital, 540 06 Thessaloniki, Greece; telephone 0030/31/994.761-2; fax 0030/31/994.916.

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PNEUMOSINUS DILATANS OF THE MAXILLARY SINUS
CASE REPORT

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A case of the rare condition pneumosinus dilatans of the maxillary sinus is presented. This is a rare differential diagnosis of a maxillary disease. The diagnostic imaging features of this condition are described, and a recommendation of views for adequately delineating this disorder is given. Only a few cases of pneumosinus dilatans of the maxillary sinus are described in the English-language literature, and the precise cause and pathogenesis remain obscure. We performed an enlargement of the maxillary ostium in order to increase the ventilation of the paranasal sinus. In the short-term follow-up, this patient has been asymptomatic. The postulated mechanism of air trapping in the sinus cavity seems to be logical in this case, because the symptoms were eliminated by surgically decompressing the sinus to overcome this valve mechanism.

KEY WORDS — aerocele, maxillary sinus, pneumocele, pneumosinus dilatans, sinus ectasia.

INTRODUCTION

Pneumosinus dilatans (PnD) may be defined as an abnormal expansion of part of the paranasal sinus, containing air only, that can cause prominent facial disfigurement and nasal obstruction, despite the fact that the sinus itself may be entirely functional.[1] The differential diagnosis of a unilateral maxillary mass includes benign and malignant mucocutaneous or mesenchymal neoplasms, inflammatory processes, mucoceles, odontogenic cysts, and fibrous dysplasia.[2-5] We present a case of PnD of the maxillary sinus and a literature review regarding the symptomatology, etiology, and treatment options.

CASE REPORT

In 1998, a 17-year-old man presented to the Department of Otorhinolaryngology of Complexo Hospitalar Santa Casa, Porto Alegre, Brazil, complaining of a left infraorbital swelling. The mass was first noticed 3 years earlier and had shown a slow progressive enlargement since that time. On examination, the swelling had a diffuse outline and a smooth surface of bonelike hardness and was not tender. The patient presented with complaints of worsening left nasal obstruction, local pain, and enlargement of the mass after exposure to sunlight. The results of intranasal examination were within normal limits. A computed tomography scan revealed a cystic enlargement of the anterior and medial walls of the left maxillary sinus, containing only air. In addition, the medial bony wall of this sinus was expanded toward the nasal septum. An air pocket anterior to the left antrum could also be observed, and this was in continuity with the lumen of the left maxillary sinus (see Figure, A). In addition, there is a suggestion of another pocket at the anterior wall of the right maxillary sinus (see Figure, B). The surgery performed was an endoscopic enlargement of the maxillary ostium, made in order to increase the ventilation of the paranasal sinus. Intrasinus exploration disclosed a lining mucosa that was normal in appearance; the sinus cavity contained only air. Histologic examination of the bone and lining mucosa obtained at this procedure disclosed no appreciable abnormalities. In 10 months of follow-up, the patient has been asymptomatic.

DISCUSSION

The benign and air-filled cystic enlargement of the paranasal sinuses has been termed pneumocele, pneumatocele, PnD, and air cyst. The entity of PnD was first recognized by Meyes[6] in 1898, but was named at that time sinus pneumatocele. In 1918, Benjamins[7] described the difference between PnD and pneumatocele. He defined PnD as a benign expansion of a paranasal sinus lying within the expanded boundaries of the sinus and containing air only, and pneumatocele as a pocket of air in a soft tissue envelope extending beyond the boundaries of the sinus, which may be a complication of PnD or several other processes. Urken et al[3] proposed a system of classification of the excessively pneumatized frontal sinus and introduced a new term, hypersinus, described as a frontal sinus that has developed beyond the upper limits of a normal frontal sinus. The sinus is aerated,

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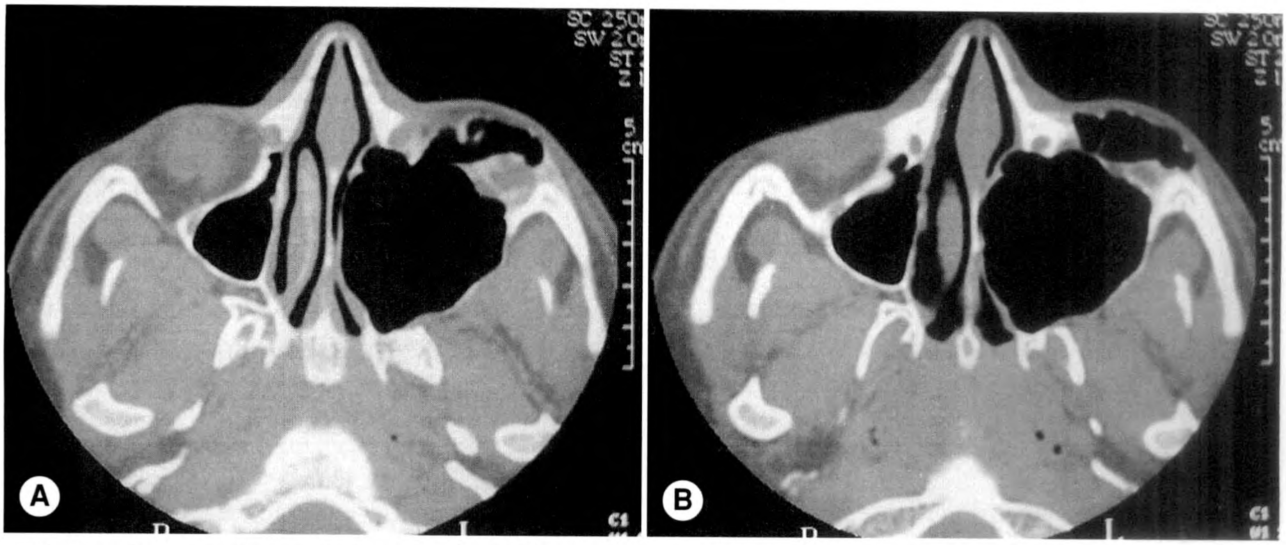

Computed tomography scan (bone window) in axial projection showing cystic enlargement of anterior wall of left maxillary sinus. A) Enlargement contains only air, and expansion of medial bony wall toward nasal septum is shown. Note continuity of left maxillary sinus with anterior air pocket. B) Small enlargement containing only air is present at anterior wall of right maxillary sinus.

with normal walls, and is larger than the usual frontal sinus; it does not extend beyond the normal boundaries of the frontal bone. Clinically, the patient is totally asymptomatic. Pneumosinus dilatans was defined as an aerated sinus that is abnormally expanded, in a focal or generalized manner, with intact and normal-thickness sinus walls that differentiate it from pneumatocele, which is an aerated sinus with either focal or generalized thinning of the bony sinus walls. In other words, the feature that differentiates PnD from pneumatocele is the loss of integrity of part, or all, of the bony sinus wall. Depending upon the degree of sinus involvement, the entire sinus cavity or only a localized portion may show abnormal expansion. Localized pneumatocele may represent a progression of PnD in which erosion or thinning of the involved bony sinus wall occurs.[3]

A review of the literature reveals that age at presentation varies from puberty to old age, but PnD has not been reported in children.[2] This may be due

also arise from the alveolar recess.[7] Other reports demonstrate diffuse maxillary enlargement.[11] Pneumosinus dilatans of the maxillary sinus may present as a slowly progressive sinus deformity that is asymptomatic[2,7,10] or is accompanied by complaints of pain or pressure over the cheek (especially upon nose blowing or sneezing), eye displacement, sinusitis,[12] proptosis, nasal obstruction,[2] eustachian tube obstruction, loss of hearing, or ear pain.[11]

The cause of primary PnD has been the source of great debate for many years. Five possible mechanisms have been proposed: a spontaneously draining mucocele, the presence of a gas-forming microorganism, the presence of a 1-way valve, a congenital malformation, or a hormonal cause.[3] The postulated mechanism of air trapping in the sinus cavity is given credence by the various reports in the literature of patients who noted an exacerbation of pressure symptoms upon airplane descent or by autoinflation upon sneezing, with elimination of these

[redacted]

the anterior wall of both maxillary sinuses. It seems that another process was going on, most likely a problem related to bone wherein new bone formation did not occur in the normal fashion of slowly continuous bone turnover. Bone tissue is made only by osteoblasts. After its formation, a further increase in size is achieved only by deposition of new bone on a preexisting surface. Primitive mesenchyme is the precursor of the earliest skeleton. Bones derived from intramembranous formation, such as cranium, are formed directly from mesenchyme. Osteoblasts and osteoclasts act in coordination and are considered the functional units of bone. The processes of bone formation and resorption are tightly coupled, and their balance determines skeletal mass at any point in time. In the formation and maintenance of the skeletal system, the osteoblast provides much of the local control, because it not only produces bone matrix, but it also plays an important role in mediating osteoclast activity.The cytokines and growth factors, especially transforming growth factor beta, released from matrix act as a feedback loop and trigger the formation and activation of osteoblasts to synthesize and deposit an equivalent amount of new bone in the resorption pit. In this fashion, bone formation and resorption are temporally and spatially related and can be controlled by systemic and local factors. Abnormalities in the control system of bone turnover, especially in bones derived from intramembranous formation, could produce a slowly continuous bone turnover.[13] This theory seems to explain the enlargement of the maxillary sinus and air pocket at the anterior antrum of both sides.

Questions remain concerning not only the cause of PnD, but also the timing of surgery and the likelihood of recurrence after surgery. A variety of surgical options have been proposed, based on a presumed causation of malfunction of the maxillary ostium. Direct sinus needle puncture has been proposed,[2,11] but an antral window made through a Caldwell-Luc exploration[2,10,11] or endoscopically would seem more definitive.[2,12] If a malfunctioning sinus ostium is suspected as the cause of the disease, then early surgery may stop the progression of deformity. On the other hand, if a different etiologic mechanism exists, then early surgery may not be of benefit. Although our patient has not developed a recurrence to date, 10 months of follow-up is too short a period to confirm success of the surgery.

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LARYNGEAL BLASTOMYCOSIS: A COMMONLY MISSED DIAGNOSIS

REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

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Blastomycosis is a relatively uncommon fungal disease that most commonly affects the lungs. Other organs may be involved, usually secondary to dissemination of the organism. Laryngeal blastomycosis may occur in isolation from active pulmonary disease. The signs, symptoms, clinical features, and pathological findings of laryngeal blastomycosis mimic those of squamous cell carcinoma. Misdiagnosis may result in inappropriate treatment with potential morbidity. Proper understanding of the clinical presentation and familiarity with the histopathologic features of this disease are therefore imperative. In this paper, we report 2 cases of laryngeal blastomycosis, 1 of which was misdiagnosed as squamous cell carcinoma, clinically and microscopically, with consequent radiotherapy and laryngectomy. In the other case, a clinical diagnosis of glottic squamous cell carcinoma was rendered. However, blastomycosis was identified in a biopsy specimen. We also review cases of *isolated* laryngeal blastomycosis that have been reported in the English-language literature during the last 80 years. A number of those cases were misdiagnosed clinically and microscopically as squamous cell carcinoma.

KEY WORDS — blastomycosis, larynx, squamous cell carcinoma.

INTRODUCTION

Blastomycosis is an uncommon though potentially serious infection in humans and has a broad spectrum of clinical disease. A soil saprophyte, *Blastomyces dermatitidis* produces airborne spores that are thought to represent the infective particles. In nature it exists in warm, moist soil rich in organic debris.[1-3] Persons exposed as a matter of occupation or recreation to soil, particularly wooded areas, are felt to be at higher risk. When inhaled, the spores undergo transformation into yeast form in the lung, and the yeast further reproduces by budding. Extrapulmonary manifestations are thought to arise by hematogenous spread from the primary pulmonary focus and most commonly include the skin, bone, and genitourinary system.[1-4] In 1894, Gilchrist[5] first described blastomycosis infection as primarily involving the skin (hence the emphasis in the name). Subsequently, the deeper and more disseminated nature of the infection was recognized.[6] Primary cutaneous inoculation blastomycosis is extremely rare and occurs exclusively at laboratory or autopsy room infection.[2]

In the absence of identifiable pulmonary or cutaneous disease, laryngeal blastomycosis can present a diagnostic challenge to both clinician and pathologist.[7-19] Typically, patients with laryngeal disease complain of insidious onset of hoarseness and occasionally pain and dysphagia of several months' duration. On examination, these lesions may be erythematous, ulcerated, and fungating, thus mimicking carcinoma.[7-19] Microscopically, the mucosal lesion often shows pseudoepitheliomatous hyperplasia that may be florid and difficult to distinguish from laryngeal squamous cell carcinoma.[8,11-13,16-18]

CASE REPORTS

Case 1. A 62-year-old chain saw operator for the timber industry in rural Kentucky presented to an outside otolaryngology service in September 1997 for symptoms of sore throat and hoarseness persisting over a period of 2 to 3 months. His past medical history was remarkable for interstitial pulmonary disease of undetermined cause. His tobacco use was 2 to 3 packs of cigarettes per day for 50 years. He admitted frequent binge alcohol consumption over the same period. His family history was remarkable for oral cancer in his mother and throat cancer in his maternal grandmother. Examination revealed an exophytic mass involving the right anterior two thirds of the true vocal cord with minimal cord mobility. No cervical adenopathy was present. The patient underwent 3 biopsies of the lesions in September, November, and December of 1997. The first 2 biopsies yielded a diagnosis of epithelial cell atypia, and the third, invasive squamous cell carcinoma. After consultation with his physician, the patient elected primary radiotherapy treatment for his larynx.

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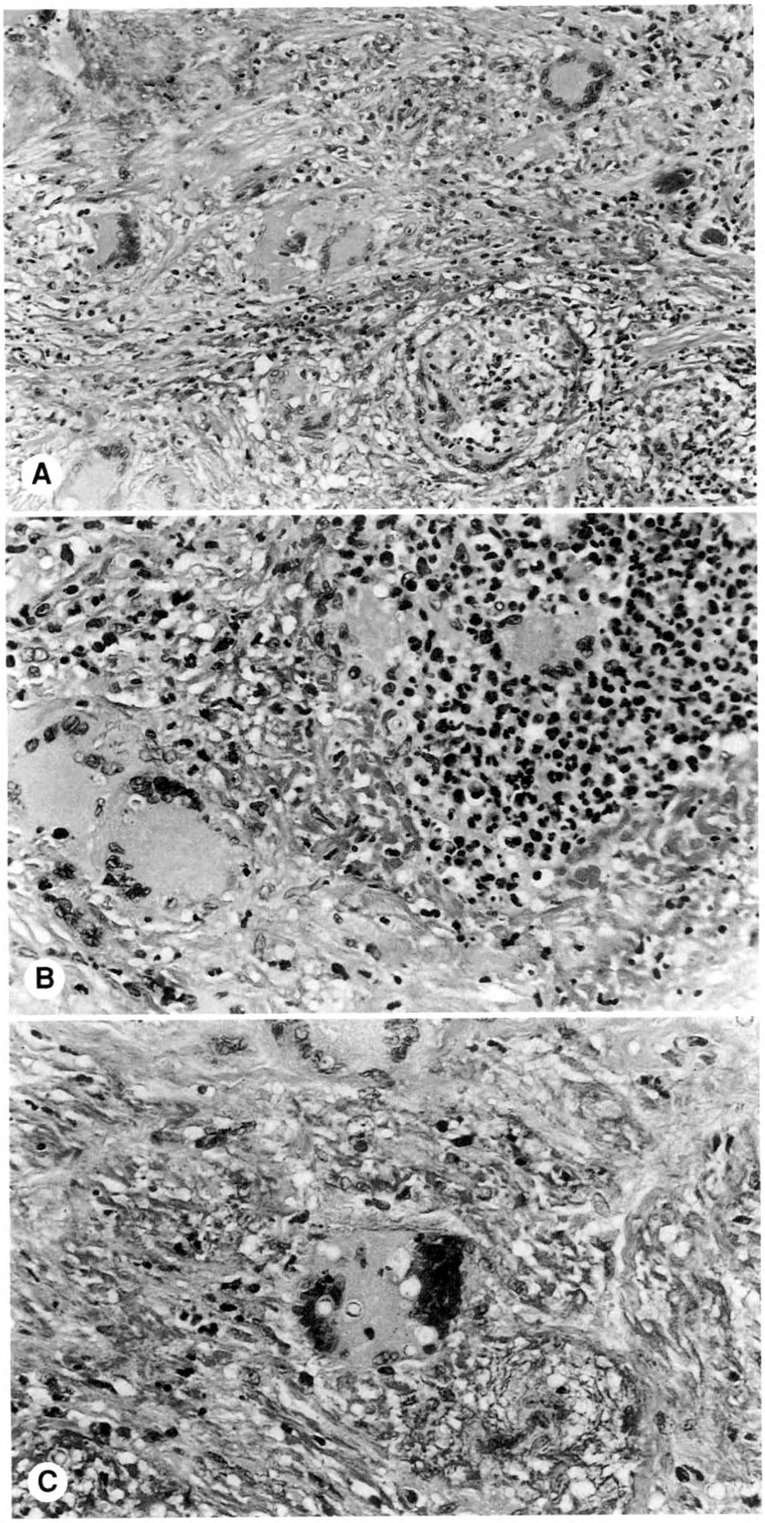

Fig 1. (Case 1, H & E) A) Granulomatous inflammation and numerous scattered multinucleated giant cells, some of which are Langhans'-type cells (upper right). B) Submucosal microabscess in proximity to several giant cells. C) *Blastomyces dermatitidis* yeast forms within giant cell.

The patient presented to our institution in June 1998 complaining of persistent throat soreness, aphonia, and continual aspiration of oral secretions. A percutaneous gastric tube had been placed, as he was unable to maintain adequate nutrition by mouth and had lost 9 kg (20 lb) since concluding radiation treatment. On examination, he appeared chronically ill and cachectic. Fiberoptic endoscopy revealed marked mucosal ulceration and underlying cicatricial changes in the epiglottis and bilateral false and true vocal cords. The true vocal cords were fixed in the abducted position. The laryngeal vestibule was filled with pooled secretions. A modified barium swallow study showed moderate silent aspiration. The risks, benefits, and reversibility of several procedures for management of aspiration were discussed with the pa-

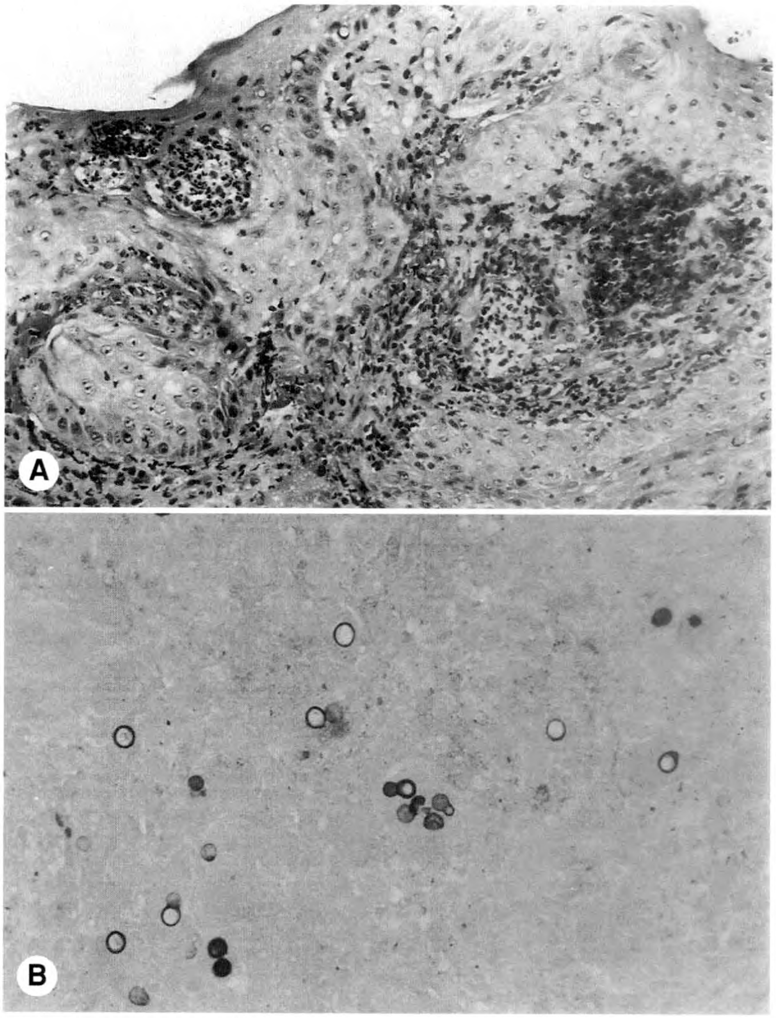

Fig 2. (Case 1) A) Pseudoepitheliomatous hyperplasia and intraepithelial microabscess formation (H & E). B) *Blastomyces dermatitidis* yeast forms showing broad-based budding (Gomori–methenamine silver).

tient. The patient chose laryngectomy.

Histologic examination of the laryngectomy specimen showed ulceration, fibrosis, and chronic granulomatous inflammation with numerous giant cells, in addition to focal microabscess formation (Fig 1). A few yeast forms consistent with *B dermatitidis* were discernible on routine microscopy within and outside giant cells (Fig 1C). A Gomori–methenamine silver stain showed numerous budding, thick-walled, yeastlike organisms of blastomyces. Sputum stains also tested positive for the organism.

A review of the preradiotherapy laryngeal biopsy also confirmed the diagnosis of blastomycosis. The sections showed acanthotic surface epithelium with pseudoepitheliomatous hyperplasia and epithelial atypia. Acute and chronic inflammatory cell infiltrates, in addition to a few granulomas, were present submucosally. Microabscesses had formed intraepithelially and submucosally (Fig 2). There was no evidence of true dysplasia or invasive malignant proliferation. Fungal stains were positive for blastomyces (Fig 2B).

The postoperative course was complicated by hypoxia and progressive alveolar infiltrate on chest x-ray films. The patient began a course of intravenous amphotericin B, 0.5 mg/kg per day (35 mg) for a total dose of 1,000 mg, and showed slow improvement. At the completion of his amphotericin B therapy, he was converted to itraconazole 20 mg orally twice per day for 12 weeks. The postoperative course was also complicated by peristomal fistula that failed to respond to conservative measures or to a pectoralis myocutaneous flap closure. The patient ultimately improved with hyperbaric oxygen therapy.

Case 2. A 45-year-old woman presented in June 1998 complaining of hoarseness, exacerbated by voice use, that had progressively worsened over the preceding 10 months. A "polyp" of the right true vocal cord had been removed 7 months previously with a minimal change of the patient's hoarseness. She also complained of mild dysphagia and a daily cough productive of minimal amounts of clear sputum. She noted mild dyspnea with exercise, but denied constitutional symptoms (fever, chills, sweats, or weight loss). ~~Her past medical history was unremarkable.~~

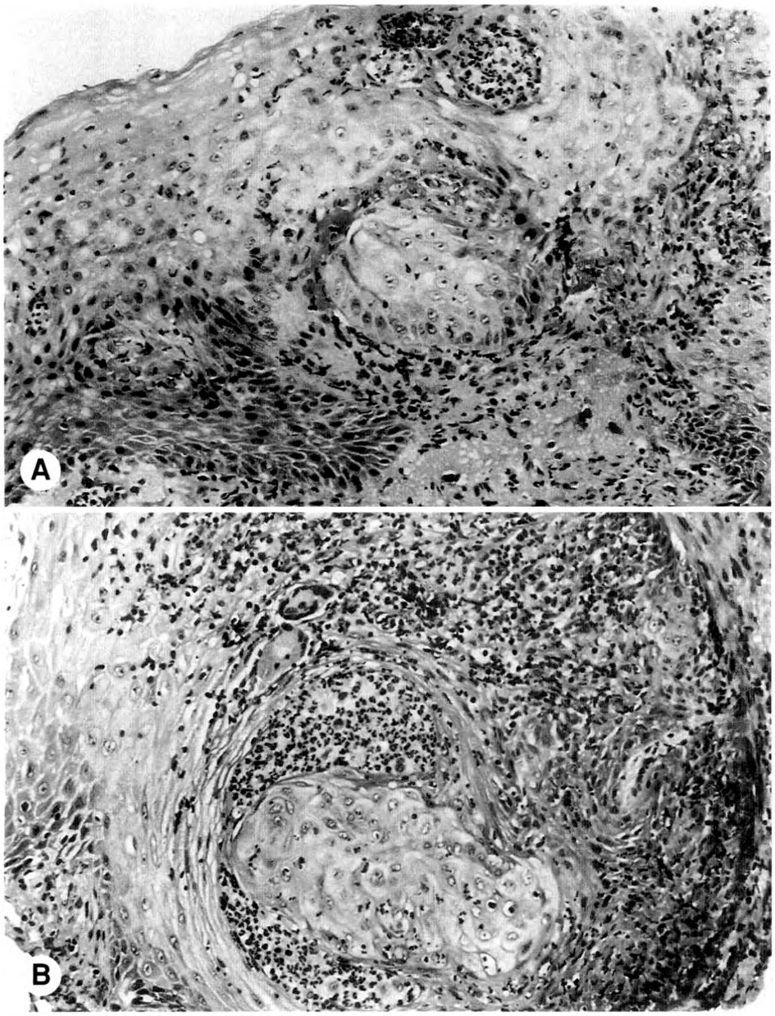

Fig 3. (Case 2, H & E) A) Pseudoepitheliomatous hyperplasia and intraepithelial microabscess formation. B) Higher magnification showing reactive epithelial atypia associated with abscess and giant cells.

She had smoked 1 to 2 packs of cigarettes per day for 30 years and admitted to occasional alcohol use. She spent weekends during the summer at a nearby lake cabin. Chest films showed left peribasal and perihilar infiltrates consistent with old, inactive infection.

On examination, an erythematous polypoid lesion of the posterior aspect of the right true vocal cord with a small amount of leukoplakia at its medial border was noted. The right cord was hypomobile. A more broadly based and bulkier erythematous polyp was noted on the left posterior true vocal cord. The remainder of the findings on head and neck examination were normal. No cervical adenopathy was noted. The initial impression was of squamous cell carcinoma of the glottis.

Biopsy of the lesion revealed granulomatous inflammation and microabscess formation. Pseudoepitheliomatous hyperplasia with epithelial cell atypia was also present (Fig 3). Gomori–methenamine silver stains showed thick-walled yeast forms of fungal organisms with occasional broad-based budding, consistent with *Blastomyces* species. A diagnosis of laryngeal blastomycosis was rendered.

The patient received intravenous amphotericin B 50 mg/d for 8 days (total dose 400 mg). She continued with itraconazole 200 mg twice per day orally for 8 weeks. On follow-up at conclusion of her therapy, the patient reported minimal residual hoarseness, and no cough or dysphagia. Her true vocal cords were normal in appearance. The right cord had persistent slight hypomobility on phonation.

DISCUSSION

In the absence of identifiable systemic disease, laryngeal lesions of blastomycosis are referred to as primary or isolated laryngeal blastomycosis. Eighteen such cases have been reported in the English-language literature during the last 80 years, several of which were in single case reports.[8-13] These cases presented a diagnostic challenge, and most were misdiagnosed clinically as carcinoma. In several instances, a malignant diagnosis was rendered microscopically as well.[11-13,16] These patients, as in case 1 of this report, were invariably subjected to radical surgery and/or radiotherapy.

The majority of patients with laryngeal blastomycosis are men ranging from 34 to 73 years of age.[7-19] The patients often present with an insidious onset of symptoms. Persistent hoarseness lasting several months is the most common complaint.[13,16] Sore throat and dysphagia may also be present. Examination usually reveals erythematous, granular exophytic changes of the laryngeal mucosa and may involve any site from the epiglottis to the subglottis. Minute grayish papules or larger yellow nodules representing small abscesses may be seen superficially.[13,16,20] Vocal cord movement may be impaired by the surrounding inflammatory processes. In advanced cases, fistulization to the anterior part of the neck may occur.

The initial pulmonary blastomycosis infection may have gone unnoticed by the patient, as it is frequently self-limited and often resolves without specific intervention. Chest radiographic findings are variable and nonspecific or may be lacking altogether.[3] Laboratory testing to confirm the diagnosis of blastomycosis is of limited usefulness. Currently available complement fixation and antigen skin tests have demonstrated poor sensitivity. A new sandwich immunoassay technique was reported to have high sensitivity and specificity, but is not commercially available.[21]

The larynx may be involved via a hematogenous route from quiescent pulmonary disease, or alternatively, fungus-laden sputum traversing the larynx may cause infection by direct inoculation.[22] Dennis,[7] in 1918, reported the first case of laryngeal involvement in the English-language literature. Over the last 80 years, laryngeal blastomycosis has been reported infrequently. In the largest otolaryngological series reported, Dumich and Neel[16] identified only 6 instances of laryngeal involvement in 107 cases of blastomycosis seen in over 30 years at the Mayo Clinic. Two of the 6 patients had no evidence of extralaryngeal involvement. Two instances of laryngeal involvement were noted in 326 cases of systemic blastomycosis reported in a 10-year collection from Mississippi.[23]

Microscopic examination of the mucosal lesion is the most reliable method for diagnosis of blastomycosis.[1,2,4] However, the histologic findings may be misleading. Essentially, there is a nonspecific inflammatory infiltrate associated with surface epithelial changes that can be confused with squamous cell carcinoma. The inflammatory infiltrate is composed of granulomas and aggregates of polymorphonuclear leukocytes. The granulomas are usually noncaseating and composed of epithelioid histiocytes and multinucleated giant cells. Dispersed giant cells may be found unassociated with well-formed granulomas. Epithelial microabscess formation is common.[1,2]

The reactive epithelial changes that are described as pseudoepitheliomatous hyperplasia are composed of downward proliferation of surface epithelium into the underlying connective tissue. Tangential sectioning of enlarged epithelial ridges produces squamous epithelial islands that, in the 2-dimensional microscopic sections, appear detached from the surface mucosa. This finding, particularly if combined with reactive epithelial atypia, may be confused with invasive squamous cell carcinoma. However, careful examination reveals that true dysplasia and invasion through the epithelial basement membrane are absent. The presence of submucosal multinucleated giant cells, granulomas, and mucosal as well as submucosal microabscesses should be a clue to the pathologist to reconsider the diagnosis of malignancy. Identification of the organism is essential. When identified by special stains, the organisms are round, thick-walled yeast cells, 10 to 15 μm in diameter, typically forming broad-based buds.[1,2,4] They may be present within or outside giant cells.

Many patients with laryngeal blastomycosis can be treated on an outpatient basis with oral itraconazole. A dose of 200 mg twice per day is given over a prolonged course of 3 to 6 months, with the duration being guided by the clinical status of the disease. Alternatively, intravenous amphotericin B given once daily for a total dose of 1 to 2 g is appropriate in patients in whom life-threatening illness supervenes or in whom compliance with a protracted outpatient regimen is in doubt.[3,18]

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GLOTTOGRAPHIC PHASE DIFFERENCE IN RECURRENT NERVE PARALYSIS

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Phase measures with simultaneously recorded electroglottography (EGG) and photoglottography (PGG) signals have been studied in canine models and found to be sensitive to the effect of recurrent laryngeal nerve (RLN) paralysis on vocal fold vibration. This study examined the usefulness of this type of measure in clinical application. The combined glottographic signals were obtained from 5 men with a diagnosis of unilateral RLN paralysis and 5 age-matched controls. In the patient group, EGG waveforms were found to have a consistent phase delay in relation to PGG. A measure of the overall glottographic phase difference (GPD) was found to significantly distinguish the control group (mean GPD = 0.2371 ms) from the patient group (mean GPD = –0.2765 ms). A validity analysis performed on 19 subjects with or without unilateral RLN paralysis confirmed that the GPD test had a relatively high efficiency (91.7%) in detecting unilateral RLN paralysis in the male population.

KEY WORDS — electroglottography, paralysis, phase analysis, photoglottography, recurrent laryngeal nerve, voice.

Glottographic measures of phonation, such as photoglottography (PGG) and electroglottography (EGG), provide physiological information closely related to the actual vocal fold movement.[1-4] Some temporal relationships within each vibratory cycle are interpretable from both EGG and PGG waveforms,[1,5-13] and abnormal patterns of glottographic signals have been detected in vocal disorders.[8,10,14-17] Observations through videostroboscopic images synchronized with glottographic signals have been used to substantiate the association between abnormalities in the glottographic waveforms and specific pathophysiological entities.[14-17] The clinical application of glottographic measures has also been explored. However, more patient statistics are needed to validate the usefulness of glottographic measures as diagnostic or assessment tools.

The PGG method uses a light source above and a light transducer below the vocal folds to record variations in transillumination corresponding to the cross-sectional area of the glottal aperture. The EGG technique involves 2 electrodes placed on opposite sides of the neck to detect electrical impedance variations that change with the extent of the vocal fold contact. Because EGG provides more information on the closed phase of a glottal cycle, whereas PGG provides more information on the open portion, the 2 methods can be simultaneously used to enhance characterization of the vibratory behaviors of the vocal folds.[8] The parameterization of glottographic waveforms has been based not only on 1 glottogram alone,[3,8,18,19] but also on synchronized glottographic signals.[5,11,20,21] Measures based on 2 combined glottographic signals provide more reference points for measuring the time relationships of glottic events. Trapp and Berke[20] observed 2 simultaneous glottic signals in a canine model. They reported that during the high-impedance plateau of least glottal contact shown in EGG, the apex of the PGG indicating maximum glottal opening occurred earlier when the condition of recurrent laryngeal nerve (RLN) paralysis was simulated. In the normal condition, the peak of the PGG waveform typically fell in the latter portion of the EGG plateau phase. The quantifier of this phenomenon was termed the shift quotient, calculated as the interval between the beginning of the EGG plateau (minimal glottal contact) and the peak of the PGG (maximum glottal opening), divided by the duration of the EGG plateau.[20] The shift quotient has been shown in canine models to distinguish the normal larynx (reported group means ranging from 0.57 to 0.65) from simulated unilateral RLN paralysis (reported group means ranging from 0.32 to 0.39) with a statistical significance,[20,21] and to reflect effects of vocal fold augmentation on laryngeal vibration in simulated RLN paralysis.[21]

A time delay of the EGG plateau relative to the peak of the PGG waveform has also been observed

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in patients with unilateral RLN paralysis.[8,20] However, the clinical significance of this type of glottographic measure has not been demonstrated. To supplement the lack of clinical data, this study examined the glottal phase relationships in glottographic signals obtained from patients with unilateral RLN paralysis and individuals with no voice disorder. The objective of this study is to develop and establish a sensitive measure of the phase difference between EGG and PGG waveforms that may be used to detect unilateral vocal fold paralysis in human subjects. From animal model studies,[20,21] we hypothesized that a lack of stiffness in 1 paralyzed vocal fold would cause an increase in the rate of glottal opening in PGG. We also hypothesized that lack of stiffness in a fold would be associated with a decrease in the glottal area of contact, thereby prolonging the plateau phase of the EGG signal. The combination of these 2 effects of paresis would result in the EGG waveform's lagging behind the PGG waveform in the open portion of a glottal cycle. Because the shift quotient as previously defined was not calculable when the peak of the PGG was shifted beyond the EGG plateau,[21] the time relationship between EGG and PGG signals was measured in this study from reference points defining 2 parallel portions in the 2 signals. We determined the relative time loci of the EGG and PGG waveforms by the peaks and nadirs in the first derivative of the 2 glottographic signals. These points of change in the first derivative of the signals indicated the maximum change of the signals in velocity at opening and closing. The peak of the differentiated EGG has been interpreted as the moment of upper margin opening, and the nadir as the moment of rapid lower margin closure.[5,9,11,20,21] The peak of the first derivative of the PGG may represent the starting point of continued lateral upper widening fold movement after complete separation of the vocal folds, and the nadir may identify the rapid narrowing of the glottal aperture.[9] Given the same mathematical derivation, these reference points consistently demarcated in both signals the dynamic open portion of a glottal cycle, even in the absence of a complete glottal closure.

This study consisted of 2 experiments: 1 involving patients with unilateral RLN paralysis and age-matched controls to generate development data, and the other involving a larger corpus of patients and controls to generate prediction data. In the first experiment, 3 glottographic measures, reflecting the time relationships between EGG and PGG during the opening, the closing, and the overall open period of a glottal cycle, respectively, were tested with the development data. In the second experiment, a computerized algorithm was developed to facilitate glottographic measurement, and a validity analysis of the measurement was performed with the prediction data.

EXPERIMENT 1

METHOD

Subjects and Subjects' Task. The subjects were 10 men, including 5 patients with diagnoses of unilateral RLN paralysis and 5 age-matched controls. The mean age of the patient group was 47.2 years (SD = 17.1), and that of the control group was 47.0 years (SD = 17.7). Each patient had documented surgical section or specific injury to the RLN, with a confirmed diagnosis of compensated RLN paresis made by examinations of the larynx. The diagnoses were made without prior knowledge of any glottographic information. The control subjects were healthy volunteers with no current or past evidence of voice or speech problems and with normal larynges. The subjects' task was to sustain the vowel /i/ at a comfortable pitch and loudness level as steadily and as long as possible while the recording devices were in place.

Instrumentation. The PGG recording device included a photosensor (Centronics OSD5-5) positioned over the neck of the subject below the cricoid cartilage and a light projected from a flexible fiberoptic nasopharyngoscope (Olympus BF-3C3) inserted through a nostril. The EGG recording device consisted of an electroglottograph (Synchrovoice Research) connected to 2 surface electrodes fixed on the sides of the neck of the subject at the thyroid alae. A condenser microphone (B & K 4144) was used for acoustic recording. A multichannel digital tape recorder (TEAC RD-180T PCM) was used to store PGG, EGG, and acoustic signals simultaneously on a digital audiotape (SONY DT-60P).

Recording Procedure. The subjects were seated upright, with the electrodes and the photosensor secured on the subject's neck. The subject was asked to perform the experimental task with the light source positioned above the supraglottal area, around the velum. Several trials were repeated to obtain a complete simultaneous recording of both glottographic signals.

Data Retrieval and Analysis. The output of the data recorder was connected to a personal computer (AST Advantage Adventure 8075P) via a 12-bit analog-to-digital converter (National Instruments AT-MIO-E-2). The output of the recorder was also connected to an oscilloscope for a simultaneous display of PGG and EGG signals. While listening to the acoustic channel and watching the display on the oscilloscope, the experimenter digitized the signals at a sampling rate of 20 kHz with a data acquisition program in LabVIEW 4.0 (National Instruments). Ten consecu-

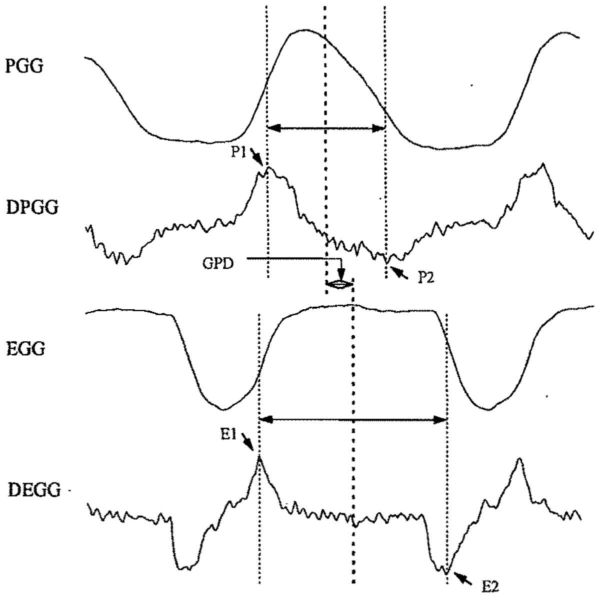

Fig 1. Illustration of marking of differentiated EGG (DEGG) and differentiated PGG (DPGG) for phase measures of glottographic signals. EGG — electroglottography, PGG — photoglottography. See text for other abbreviations.

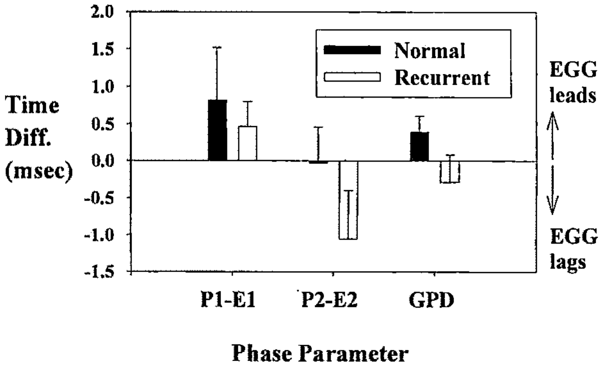

Fig 2. Comparison between normal group and patient group on mean and standard deviation measures of 3 phase parameters.

tive cycles of the digitized PGG and EGG signals in the midportion of a sustained vowel phonation were segmented from 1 representative trial for each subject. The signals were imported to CSpeech 4.0 (author, P. H. Milenkovic) for waveform display, differentiation, and measurement.

The simultaneously recorded PGG and EGG waveforms were displayed so that an increase of the PGG signal represented the increase of glottal transillumination and an increase of the EGG signal represented the increase of glottal impedance. From each signal, the first derivative was calculated and displayed simultaneously in time. The maximum peak and valley in each cycle of the differentiated waveforms, shown in Fig 1 as points P1, P2, E1, and E2, were hand-marked by an experimenter moving a cursor along the waveforms. The time values of these points were entered into SigmaPlot 3.0 and SigmaStat 2.0 (Jandel Scientific) for transformation. Three glottographic time differences were calculated: 1) the glottographic time difference in the opening stage (P1 – E1), 2) the glottographic time difference in the closing stage (P2 – E2), and 3) the overall glottographic phase difference (GPD), which was the average of the previous 2 time differences and could also be calculated by subtracting the time value of the midpoint between E1 and E2 from that between P1 and P2. A negative GPD indicated that EGG lagged behind PGG.

Reliability. Measurement reliability was assessed by another experimenter's remeasuring 56% of the data for the GPD values. A high measurement reliability was indicated by the result of a Pearson correlation between the 2 sets of independently measured data (r = .996, p < .001).

RESULTS

Table 1 summarizes results for the 3 phase measures, the P1 – E1 time difference, the P2 – E2 time difference, and the GPD measure. Figure 2 illustrates comparisons of the means and standard deviations between normal and patient groups on these 3 phase measures. Figure 2 shows that for both subject groups, the EGG signal leads the PGG signal in the opening stage, but lags behind the PGG signal in the closing stage. Figure 2 also shows that the mean GPD value in the control group is positive, while that in the patient group is negative, indicating that the overall EGG signal tends to lead the PGG in the normal group, but follows behind the PGG signal in the pa-

TABLE 1. AGE AND VALUES ON THREE HAND-MEASURED GLOTTOGRAPHIC PHASE PARAMETERS FOR FIVE PATIENTS AND FIVE AGE-MATCHED CONTROLS

Controls					*Patients*				
Subject	*Age*	*P1 – E1 (ms)*	*P2 – E2 (ms)*	*GPD (ms)*	*Subject*	*Age*	*P1 – E1 (ms)*	*P2 – E2 (ms)*	*GPD (ms)*
1	27	0.85 ± 0.41	–0.32 ± 0.14	0.27 ± 0.22	6	28	0.36 ± 0.09	–2.15 ± 0.36	–0.90 ± 0.19
2	30	0.91 ± 0.14	–0.19 ± 0.06	0.36 ± 0.08	7	31	0.68 ± 0.15	–0.91 ± 0.42	–0.12 ± 0.21
3	52	–0.14 ± 0.02	0.85 ± 0.76	0.36 ± 0.38	8	52	0.48 ± 0.24	–1.07 ± 0.13	–0.30 ± 0.14
4	59	1.82 ± 0.23	–0.30 ± 0.15	0.76 ± 0.15	9	58	0.85 ± 0.78	–0.62 ± 0.70	0.12 ± 0.40
5	67	0.64 ± 0.34	–0.21 ± 0.05	0.22 ± 0.17	10	67	–0.03 ± 0.62	–0.52 ± 0.13	–0.27 ± 0.28

Values are mean ± SD. See text for abbreviations.

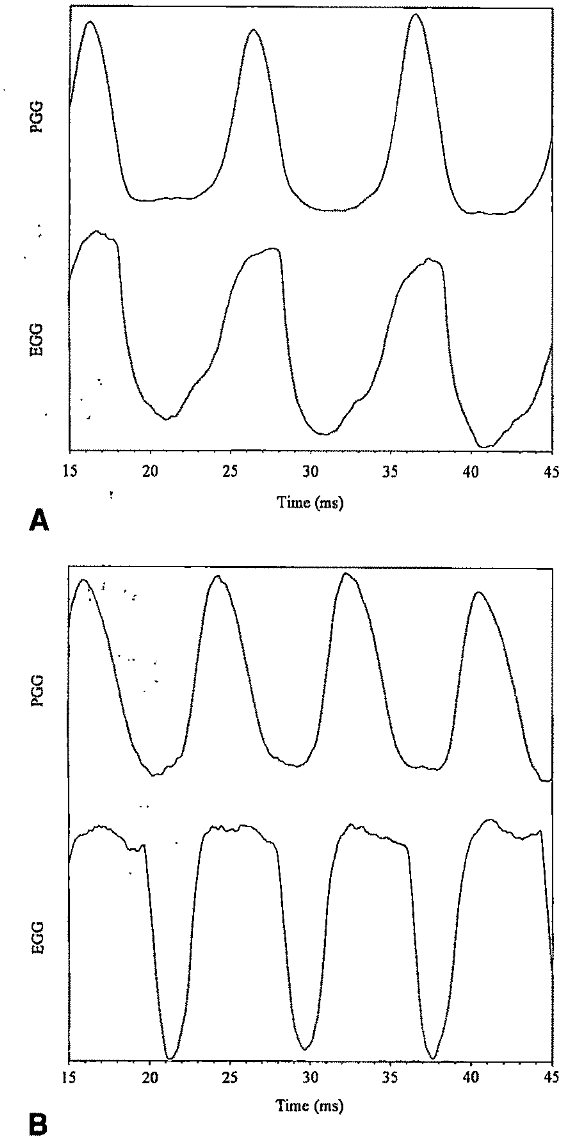

Fig 3. Synchronized displays of PGG and EGG waveforms for A) normal speaker and B) patient with recurrent laryngeal nerve paralysis.

tient group. The same finding can be observed from Fig 3, showing a synchronized display of the EGG and PGG for a normal subject and for a patient with RLN paralysis. Results of a series of multiple *t*-tests with a Bonferroni correction comparing the 2 subject groups on the 3 phase measures revealed that only the GPD measure significantly differentiated the normal group from the patient group ($df = 8$, $t = 3.549$, $p = .008$). The 2 subject groups were not significantly different in the measures of P1 − E1 time difference ($df = 8$, $t = 1.008$, $p = .343$) or P2 − E2 time difference ($df = 8$, $t = 2.795$, $p = .023$). It was evident that measures of the glottographic phase difference in either the opening or closing stage were not as sensitive as those of the overall glottographic phase difference in differentiating the normal group from the patient group.

To investigate the effect of fundamental frequency on the GPD measure, the fundamental frequencies of the controls and patients were examined. The mean fundamental frequency value for the patient group (mean = 132 Hz, SD = 27.2) was found to be slightly higher than that of the control group (mean = 115 Hz, SD = 27.9). However, the result of a Pearson correlation between the GPD and fundamental frequency values for all subjects showed that the 2 measures were not significantly correlated ($r = -.339$, $p = .339$).

DISCUSSION

This experiment has demonstrated with human subjects that the measure of glottographic phase difference is sensitive to the effect of unilateral RLN paralysis, as previously discovered in animal investigations. Further, this experiment has shown that even in the absence of a complete glottal closure, a dynamic open portion of a glottal cycle can be defined by the 2 points of maximum velocity changes in the EGG and PGG signals and used to quantify the time relationship between the 2 glottographic waveforms. It has been pointed out in previous studies that the starting point of vocal fold opening during a glottal cycle is hard to determine in PGG signals when the opening is gradual, with an increased vertical phase difference between the upper and lower portions of the vocal folds or with the absence of a complete glottal closure.[2] The initial rise of the EGG impedance, often used to denote the initial separation of the lower fold margins, was also found to be difficult to discern when there was a gradual rise of the EGG.[5,11] The present finding suggests that the GPD measure, which was derived from combined glottographic signals, may be a powerful tool for monitoring vocal fold vibration in disorders in which normal glottal closure is disrupted. To determine the clinical usefulness of the GPD measure in detecting unilateral RLN paralysis, the predictive power of the GPD measure was evaluated in the second experiment. Some interpretations of the phase shift observed in this experiment for the patients with unilateral RLN paralysis are given below.

The finding that the dynamic open portion reflected by the EGG signal lagged behind that reflected by the PGG signal may be attributed to a combination of the lack of a complete glottal closure and the asymmetry of the vocal folds in patients with RLN paralysis. Based on the assumption of a symmetric glottis, vocal fold modeling has demonstrated in computer simulation of glottographic waveforms a close correspondence between glottal area and contact area, with the positive changes of the glottal area synchronized with the negative changes of the vocal

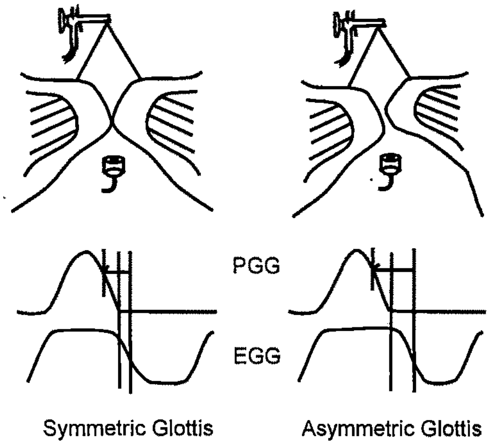

Fig 4. Coronal view of vocal fold activity during closing stage of phonatory cycle with symmetric glottal aperture and asymmetric glottal aperture and corresponding hypothesized glottographic waveforms.

fold contact.[1] It was found in the computer model, however, that there was a shift of the glottal displacement curve associated with asymmetric muscle tension, simulating unilateral RLN paralysis.[22] From both excised larynx data and computerized mucosal wave models, it has been observed that the phase relationship between PGG and EGG was related to the direction, amplitude, and phase of the traveling waves on both sides of the larynx.[9] An asymmetric traveling wave has been found, by means of videostroboscopic images synchronized with glottographic waveforms, to be one of the characteristics of the vocal fold vibratory behavior in unilateral RLN paralysis.[17] It has been speculated that the upper and lower margins of the paralyzed fold may be more tightly coupled[14] and that the difference in elasticity between the 2 vocal folds leads to a loss of synchrony. Therefore, the typical phase relationship between glottal opening (PGG) and contact area (EGG) observed in a symmetric glottis may be disrupted.

It was not surprising that during the opening stage of a glottal cycle, for both controls and patients with unilateral RLN paralysis, the P1 point tended to lag behind the E1 point, yielding a positive P1 – E1 value, because the continued lateral widening of the glottal aperture (P1) was expected to occur after complete separation of the upper fold margin (E1). The finding that the P1 – E1 time difference was shorter in the patient group, although not significantly, may have reflected the restricted lateral fold movement often observed in patients with unilateral RLN paralysis.[14] During the closing stage, it was shown in the patient group that the rapid narrowing of the glottal aperture (P2) tended to lead the moment of lower margin closure (E2). As illustrated in Fig 4, during the closing stage, the glottal aperture is closed to light transmitted parallel to the axial axis of the glottis once the lower folds have contacted each other. At this time, while the PGG signal reaches the minimum, typically, the glottal impedance shown in EGG starts to decrease, because vocal fold contact progresses. In the condition of an asymmetric glottis, however, the point of vocal fold contact is delayed, due to a loss of synchrony between the upper and the lower edges of the vocal folds. Consequently, the largest impedance change to the negative in EGG is delayed relative to the PGG signal in patients with unilateral RLN paralysis. The present interpretations of the experimental results were induced from findings based on theoretical and canine models, as well as videostroboscopic observations made in previous studies. Before these interpretations could be further verified with measurement from videostroboscopic images synchronized with glottographic signals, we focused in the second experiment of this study on the validity test of the GPD measure.

EXPERIMENT 2

METHOD

Subjects and Subjects' Task. Nineteen adults, ranging in age from 22 to 61 years of age (mean = 37.9 years; SD = 12.4), served as subjects. These subjects included 8 patients (2 women and 6 men) with unilateral RLN paralysis and 11 normal speakers (5 women and 6 men). These subjects underwent the same examination procedure and performed the same task as those of the first experiment.

Instrumentation and Recording Procedure. The instrumentation was essentially the same as that in the first experiment, with slight modifications. The PGG recording device included a custom-made photosensor positioned over the neck of the subject, below the cricoid cartilage, and a light projected from a rigid endoscope (Richard Wolf) inserted through the mouth. The EGG recording device consisted of an electroglottograph (Glottal Enterprise) connected to 2 surface electrodes fixed on the sides of the neck of the subject at the thyroid alae. A condenser microphone (Radio Shack) with a custom-made amplifier was used for acoustic recording. The outputs of the EGG, PGG, and acoustic devices were connected either to a personal computer (AST Advantage Adventure 8075P) via a 12-bit analog-to-digital converter (National Instruments AT-MIO-E-2) for direct digitization or to a multichannel digital tape recorder (TEAC RD-180T PCM) for tape recording onto a digital audiotape (SONY DT-60P).

Data Retrieval and Analysis. In this experiment, 37% of the data were directly digitized during the signal recording procedure, and 63% of the data were retrieved from the digital tape and digitized. The digi-

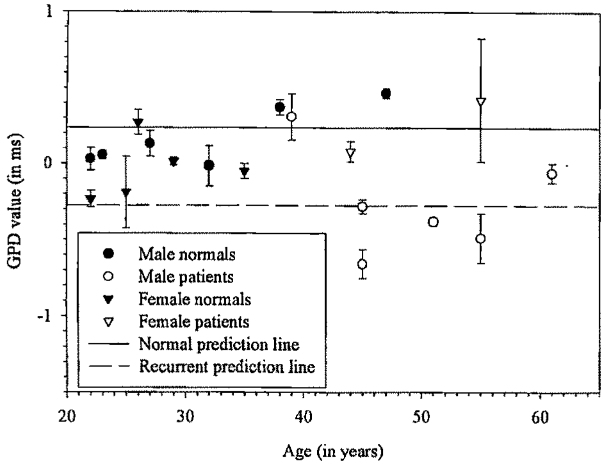

Fig 5. Individual mean glottographic phase difference (GPD) values (standard deviation in error bar) for 19 subjects used to test predictive power of GPD measure. Two reference lines (normal = 0.2371 ms; recurrent laryngeal nerve paralysis [recurrent] = –0.2765 ms) are group means obtained from 5 patients and 5 age-matched controls used as development data.

tization procedure was the same as that described in the first experiment. The measurement in this experiment was automated with a locally developed algorithm. The signal length used to derive the mean GPD value for each of the 19 subjects varied from 3 to 101 cycles (mean = 51.7, SD = 36.4). The program low-passed both EGG and PGG signals at a frequency of 1.5 kHz and processed the 2 synchronized signals with the same differentiation and marking procedure as described in the first experiment. The program displayed the original, the filtered, and the differentiated waveforms and the marking and calculation results. The mean GPD values derived from individual subjects were imported into SigmaStat 2.0 (Jandel Scientific) for statistical analysis.

Reliability. The computerized algorithm demonstrated 100% accuracy for repeated measures of a signal. The reliability of the computerized measurement was assessed by remeasuring the data hand-measured in the first experiment with the automated algorithm. Despite a slight difference in signal length between the signals used in the first experiment (each with exactly 10 cycles) and those used in the second experiment (mean = 9.3 cycles; SD = 2.2) for the development data, a high measurement reliability was indicated by a high Pearson correlation between the hand-measured and the automatically detected GPD values (r = .95, p < .001).

Validity. The development data (data from the 5 patients and 5 controls used in the first experiment) reprocessed with the automated algorithm were used to set the criterion for judging the prediction data (data from the 19 subjects used in the second experiment). In the development data, the mean GPD value for the normal group was 0.2371 ms (SD = 0.2004), and that for the patient group was –0.2765 ms (SD = 0.2193). Each of the 19 subjects was identified as "normal" or "patient" on the basis of the proximity of the individual mean GPD value to these 2 group means. For example, a subject with a mean GPD value closer to 0.2371 ms than to –0.2765 ms was identified as "normal."

TABLE 2. VALIDITY ANALYSIS OF GPD MEASURE

	Men Only (n = 12)	*Men and Women (n = 19)*
Total number of patients (P)	6	8
Total number of normals (N)	6	11
True positives (a)	5	5
False positives (b)	0	3
Positive identifications (a + b)	5	8
Positive predictive power (a/a + b)	100%	62.5%
Sensitivity (a/P)	83.3%	62.5%
True negatives (c)	6	8
False negatives (d)	1	3
Negative identifications (c + d)	7	11
Negative predictive power (c/c +d)	85.7%	72.7%
Specificity (c/N)	100%	72.7%
Correct identifications (a + c)	11	13
Identifications made (a + b + c + d)	12	19
Efficiency [(a + c)/(a + b + c + d)]	91.7%	68.4%

The performance of the GPD measure in detecting RLN paralysis was assessed by tabulating the number of patients identified as "patient" (true positives), the number of normal subjects identified as "normal" (true negatives), the number of normal subjects mistaken as "patient" (false positives), and the number of patients mistaken as "normal" (false negatives). The sensitivity of the GPD test was calculated as the percentage of the patients correctly identified as "patient" (true positives per total number of patients), and specificity was the percentage of the normal subjects correctly identified as "normal" (true negatives per total number of normals). The positive predictive power was the ratio of true positives to the total number of positive identifications. The negative predictive power was the ratio of true negatives to the total number of negative identifications. The efficiency of the GPD test was calculated as the percentage of correct identifications. Because the judgment criterion was derived from male subjects only, the validity analysis of the GPD test was applied to the prediction data with and without female subjects separately.

RESULTS AND DISCUSSION

Figure 5 shows the mean GPD values and standard deviations derived from each of the 19 subjects.

Table 2 summarizes results for the validity analysis of the GPD measure, with and without female subjects. Both the sensitivity and specificity of the GPD test were relatively high with the male population. This finding confirms that the GPD measure is useful in detecting unilateral RLN paralysis in male individuals. The overall efficiency of the GPD test was decreased, however, from 91.7% to 68.4%, when female subjects were included. This finding suggests that there may be a gender difference in the effect of unilateral RLN paralysis on the GPD measure. The judgment criterion set in this study was based on male subjects and may not be applicable to female subjects. More data are needed to explore this gender difference and determine the usefulness of the GPD measure in detecting unilateral RLN paralysis in the female population.

CONCLUSION

The phase relationship between PGG and EGG signals was found to be powerful in detecting unilateral RLN paralysis in the male population, with the overall EGG signal lagging behind the PGG signal in the patient group.

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DOXORUBICIN CHEMOMYECTOMY: EFFECTS ON EVOKED VOCAL FOLD TENSION AND MUCOSAL WAVE

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Chemomyectomy of the thyroarytenoid muscle is a potential alternative approach to the management of spasmodic dysphonia (laryngeal dystonia) that could provide a prolonged response. To be useful, chemomyectomy should produce weakening of vocal fold closure without disruption of the mucosal wave. Sixteen dogs were studied. In 8 animals, doxorubicin hydrochloride (3 mg) and verapamil hydrochloride (0.5 mg) were injected unilaterally into the thyroarytenoid muscle 2 months before evaluation. The remaining animals served as noninjected controls. Injection of doxorubicin and verapamil decreased the average evoked tension of the vocal fold by 74.7%, compared to an average side-to-side difference of 12.7% in the control group ($p = .001$). A mucosal wave was recognized bilaterally with videostroboscopy in all dogs. Doxorubicin did not significantly change the vocal fold appearance or mucosal wave amplitude. These results support further laboratory study of chemomyectomy as a potential alternative treatment for laryngeal dystonia.

KEY WORDS — doxorubicin, larynx, spasmodic dysphonia, vocal fold.

INTRODUCTION

Idiopathic spasmodic dysphonia (laryngeal dystonia) is a focal dystonia affecting the intrinsic laryngeal muscles. Laryngeal dystonia, like other dystonias, is the result of a neurologic disorder of central motor processing characterized by involuntary, repetitive or sustained muscle contractions.[1] Systemic pharmacotherapy has resulted in little relief of symptoms.[2]

In 1976, Dedo[3] described a dramatic reduction of vocal spasms in patients with laryngeal dystonia after unilaterally sectioning the recurrent laryngeal nerve (RLN). The initial enthusiasm for this technique was tempered by a long-term follow-up study by Aronson and DeSanto.[4] In some patients, symptom recurrence after RLN section is associated with spontaneous reinnervation of the paralyzed vocal fold.[5] The temporary success of RLN section demonstrated that the symptoms of laryngeal dystonia could be overcome by directing treatment to the peripheral laryngeal neuromuscular unit.

Botulinum neurotoxin type A (Botox) injection into the vocalis muscle is the treatment of choice for adductor laryngeal dystonia.[6] Botox is also effective in selected cases of abductor laryngeal dystonia.[6] The toxin causes paralysis at the injection site by binding to the presynaptic cholinergic nerve terminal and inhibiting release of vesicle-bound acetylcholine. This process results in a long-term blockade of the neuromuscular junction and leads to changes similar to surgical denervation. These changes include muscle paralysis, atrophy, and electromyographic abnormalities.[6] Treatment success with Botox has demonstrated that only the thyroarytenoid muscle needs treatment to provide symptom relief for adductor laryngeal dystonia.

The clinical effects of botulinum toxin are of limited duration. The symptoms of laryngeal dystonia return after an average of 3 months. Return of neuromuscular action is associated with sprouting of nerve terminals and formation of new neuromuscular junctions. Because the beneficial effect of Botox is temporary, repeated injections are required.

The search for a therapeutic alternative to Botox continues for 2 reasons. First, a method to selectively decrease the activity of the thyroarytenoid muscle for longer periods of time is desirable. Both RLN section and Botox injection weaken the vocalis muscle by acting proximal to the neuromuscular junction. Return of muscle activity limits the benefits of both treatments. Second, a small number of patients do not respond adequately to Botox injections, and an alternative therapy would be beneficial. Doxorubicin is a chemotherapeutic agent with myotoxic and neurotoxic effects that could reduce vocal fold tension by acting both proximal and distal to the neuro-

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From the Department of Otolaryngology, Hennepin County Medical Center (both authors), and the Department of Otolaryngology, University of Minnesota (Goding), Minneapolis, Minnesota. Supported by the Minnesota Medical Research Foundation. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Minnesota Medical Research Foundation.

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muscular junctions of the thyroarytenoid muscle. The present study examines the effect of an intramuscular injection of doxorubicin on thyroarytenoid muscle tension and on the propagation of a mucosal wave in dogs.

MATERIALS AND METHODS

Sixteen dogs were evaluated in this study. The study was reviewed by the Minneapolis Medical Research Foundation and found to be in compliance with *The Principles of Laboratory Animal Care*, formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals*, prepared by the National Academy of Sciences. Eight dogs served as controls. The 8 dogs in the experimental group underwent direct laryngoscopy and unilateral doxorubicin injection 2 months before data collection. Direct laryngoscopy was performed after premedication with acepromazine maleate and anesthesia induction with halothane by mask only. Three milligrams of doxorubicin hydrochloride and 0.5 mg of verapamil hydrochloride were injected into 1 vocal fold under direct visualization. Injection of the left or right vocal fold was randomly determined for each animal. The 8 experimental animals were recovered and regularly observed for 2 months. The 8 animals in the control group did not undergo laryngoscopy, vocal fold injection, or 2-month observation before data collection.

Evoked Vocal Fold Tension. The animals were premedicated with acepromazine, mask-ventilated with halothane to effect, and intubated. Anesthesia was maintained with halothane. A midline neck incision was made from the anterior larynx to the sternal notch. A low tracheotomy was performed above the level of the sternal notch, and a Shiley No. 5.0 cuffed tracheal tube was inserted in the distal trachea for ventilation and anesthesia administration. A plastic disk was secured to the laryngeal surface of the epiglottis through the anterior cervical skin to retract the epiglottis. The RLNs were exposed bilaterally and fitted with a cuffed bipolar stimulating electrode (model 50-6873, Harvard Bioscience). The electrode placement was verified by observation of vocal fold movement following electrical stimulation. The nerve stimulation thresholds were determined by finding the minimal amperage (single pulse, 0.1-millisecond duration) producing consistent vocal fold movement as seen through a laryngoscope.

A second tracheotomy was performed approximately 10 cm below the cricoid cartilage and proximal to the previously placed tracheal tube. A Miller-Abbott tube (Rusch Inc, Duluth, Ga) was inserted through the second tracheotomy with the balloon placed between the vocal folds. The tube was secured in place, and the balloon was inflated with saline. The pressure inside the balloon was measured in millimeters of mercury with a pressure transducer connected to a Hewlett Packard pressure monitor. Computerized data acquisition was achieved with MacADios hardware and software (model 411, GWI Institute, Cambridge, Mass). Recurrent laryngeal nerve stimulation was performed at 3 times the threshold amperage with a 0.1-millisecond pulse width at 60 Hz for a duration of 2 seconds. Individual stimulation of each RLN was followed by bilateral RLN stimulation. This procedure (right-left-bilateral) was repeated for a minimum of 3 stimulation trials. Evoked vocal fold tension was defined as the pressure developed within the balloon during RLN stimulation.

Videostroboscopy. The Miller-Abbott tube was replaced by a Shiley No. 5.0 cuffed tracheal tube with the tip positioned rostrally below the vocal folds. The cuff was inflated to seal the upper trachea. Approximation of the cricoid and thyroid cartilages was accomplished with 2-0 Prolene suture. Humidified oxygen, warmed to 27°C, was delivered to the vocal folds via the superior tracheotomy tube at approximately 5 L/min. The RLNs were stimulated bilaterally at 2 times threshold with a 0.1-ms pulse width at 60 Hz. Minor modifications of RLN stimulation amplitude and oxygen flow were allowed to produce optimum phonation. A mucosal wave was examined with a videostroboscopy unit (model 4914, Bruel & Kjaer, Denmark) and recorded on videotape.

Upon completion of data collection and videostroboscopy, the animals were euthanized with a 40-mEq intracardiac bolus of potassium chloride while under anesthesia.

Data Analysis. The evoked vocal fold tension was estimated by the intraglottic pressure recorded by the intralaryngeal balloon during RLN stimulation. The rise in intraglottic pressure in millimeters of mercury over baseline pressure for right and left RLN stimulation during 3 stimulation trials in each animal was averaged. Subtle differences in tracheal size, balloon position within the trachea, and the amount of saline within the balloon resulted in differences in the baseline pressure. The average baseline pressure of 51 ± 15 mm Hg in the control group was similar to the baseline pressure in the experimental group (64 ± 22 mm Hg), but with a "weak trend" ($p = .22$) toward lower values. Due to the range of baseline pressures, it was felt that absolute measures of pressure could not be relied upon between animals. The stimulation protocol (right-left-bilateral = 1 set), and the averaging of 3 successive stimulation sets,

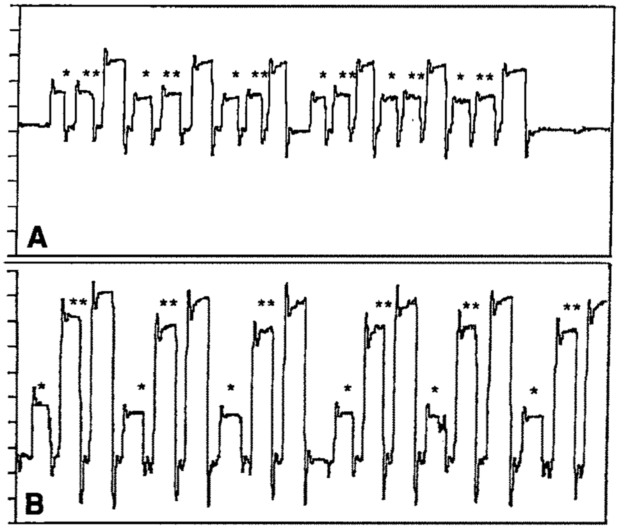

Fig 1. Sample tracings from control and injected animals used to calculate evoked vocal fold tension. Y-axis calibration marks represent 10 mm Hg. A) Tracing from control animal with intralaryngeal balloon pressure changes accompanying recurrent laryngeal nerve (RLN) stimulation following right (*)–left (**)–bilateral repetitive pattern. Baseline is at 50 mm Hg. Note similar pressure changes following unilateral RLN stimulation. B) Tracing from animal 2 months after doxorubicin injection into right vocal fold with intralaryngeal balloon pressure changes accompanying RLN stimulation following right (*)–left (**)–bilateral repetitive pattern. Baseline is at 100 mg Hg. Note difference in pressure changes between right and left unilateral RLN stimulation.

resulted in a stable baseline for measurements between the 2 vocal folds in each animal. Because of the variability between animals in regard to baseline pressure, no comparison was made between the absolute differences between the groups. Instead, a statistical comparison was made of the percent difference in evoked vocal fold tension, which is not affected by the absolute height of the measurement. The percent difference in evoked vocal fold tension was calculated by taking the difference between the 2 sides, dividing by the greater tension measurement, and multiplying by 100. Pressure measurements from the control group were analyzed for differences between the stronger and weaker vocal folds, rather than the right and left vocal folds, to bias the data against a possible treatment effect. Pressure measurements from the treatment group were analyzed for differences between the injected and noninjected vocal folds.

The videostroboscopic recordings were coded and randomly arranged on videotape. Three reviewers familiar with videostroboscopy and with no prior involvement in the study were each given a separate copy of the videostroboscopic recordings. The reviewers were told that some animals had been unilaterally injected with doxorubicin and that other animals served as controls. The reviewers were asked to first judge vocal fold appearance. Each vocal fold was individually rated as normal or abnormal. Next, the presence or absence of a mucosal wave was determined for each vocal fold. The symmetry of the mucosal wave was rated on a 5-point scale (1 = excellent symmetry, 5 = no symmetry). A 5-point scale was also used to rate the mucosal wave amplitude for each vocal fold (1 = no movement, 3 = width of the vocal ligament, 5 = width of the entire vocal fold). Finally, each reviewer was forced to decide whether the animal had received an injection, and on which side. The 2 reviewers with responses showing the greatest correlation were used to determine the final response. In cases of disagreement, the response from the third reviewer was used to break the tie.

TABLE 1. AVERAGE EVOKED VOCAL FOLD TENSIONS AND AVERAGE DIFFERENCE AND PERCENT DIFFERENCE BETWEEN PAIRS

	Evoked Vocal Fold Tension (mm Hg)	*Average Difference (mm Hg)*	*Average Percent Difference**
Control group		2.7 ± 2.6	12.7
Stronger fold	21.3 ± 7.8		
Weaker fold	18.6 ± 6.7		
Treatment group		26.8 ± 7.7	74.7
Noninjected fold	35.9 ± 11.8		
Injected fold	9.1 ± 5.5		

Data are mean ± SD.

*Difference between larger and smaller average vocal fold measurement divided by larger measurement and multiplied by 100.

RESULTS

All animals in the treatment group tolerated the injection of doxorubicin and verapamil without evidence of airway compromise or feeding difficulty. Evoked vocal fold tension was measured in 7 control and 8 treatment animals. Videostroboscopy was performed in all 16 animals.

Evoked Vocal Fold Tension. Sample tracings from a control animal and an injected animal are presented in Fig 1. The difference in average evoked tension between the stronger and weaker vocal folds in the control group, regardless of side, reveals an average difference of 2.7 ± 2.6 (SD) mm Hg (Table 1). Doxorubicin and verapamil injection into a vocal fold reduced the evoked tension by an average of 26.8 ± 7.7 mm Hg when compared to the opposite, noninjected vocal fold. The average percent difference in evoked vocal fold tension between the stronger and weaker sides was 12.2% ± 8.6% in the control group. Vocal fold injection resulted in a 76.0% ± 11.4% decrease in evoked vocal fold tension. The change within the treatment group was greater than the maximal side-to-side variation in the control (p = .001, Stu-

TABLE 2. AVERAGE STIMULATION THRESHOLDS AND AVERAGE DIFFERENCE BETWEEN PAIRS

	Stimulation Threshold (mA)	*Average Difference (mA)*
Control group		0.04 ± 0.13
Left fold	0.46 ± 0.21	
Right fold	0.41 ± 0.17	
Treatment group		0.19 ± 0.30
Noninjected fold	0.31 ± 0.24	
Injected fold	0.50 ± 0.46	

Data are mean ± SD.

TABLE 3. SUMMARY OF VIDEOSTROBOSCOPIC FINDINGS

Criteria	*Treatment*	*Control*	*p*
Abnormal appearance of individual vocal fold[a]	3/8 (38%)	6/24 (25%)	.65[b]
Presence of mucosal wave	8/8 (100%)	8/8 (100%)	
Average symmetry rating	2.6	2.2	.23[c]
Correct estimate of injection	5/8 (63%)	3/8 (38%)	.62[b]
Individual assessment of site of injection[d]	13/24 (54%)	13/24 (54%)	

[a] Sixteen total animals × 2 vocal folds per animal.

[b] Two-tailed Fisher's exact test.

[c] Unpaired Student's *t*-test.

[d] Three reviewers × 16 animals.

dent's *t*-test).

Recurrent Laryngeal Nerve Threshold. To determine if the doxorubicin injection of the vocal fold also affected the RLNs, the stimulation thresholds (milliamperes) were compared bilaterally (Table 2). The mean difference of 0.15 between the 2 groups was not significant (p = .2762, 1-factor analysis of variance). No adverse affect on the stimulation threshold of the RLN was demonstrated.

Videostroboscopy. Vocal fold appearance was judged to be normal in 5 of the 8 control animals and 4 of the 8 experimental animals (Table 3). An example of vocal fold appearance for a control animal and a treated animal is presented in Fig 2. Rating each vocal fold separately (16 total animals × 2 vocal folds per animal), 3 of 8 (38%) of injected and 6 of 24 (25%) of noninjected vocal folds were rated as having an abnormal appearance. Application of a 2-tailed Fisher's exact test to these findings showed no difference in appearance between injected and control vocal folds (p = .65).

A mucosal wave was judged to be bilaterally present in all control and injected animals. The average symmetry rating of 2.2 for control animals was similar to the 2.6 average rating for injected animals (p =

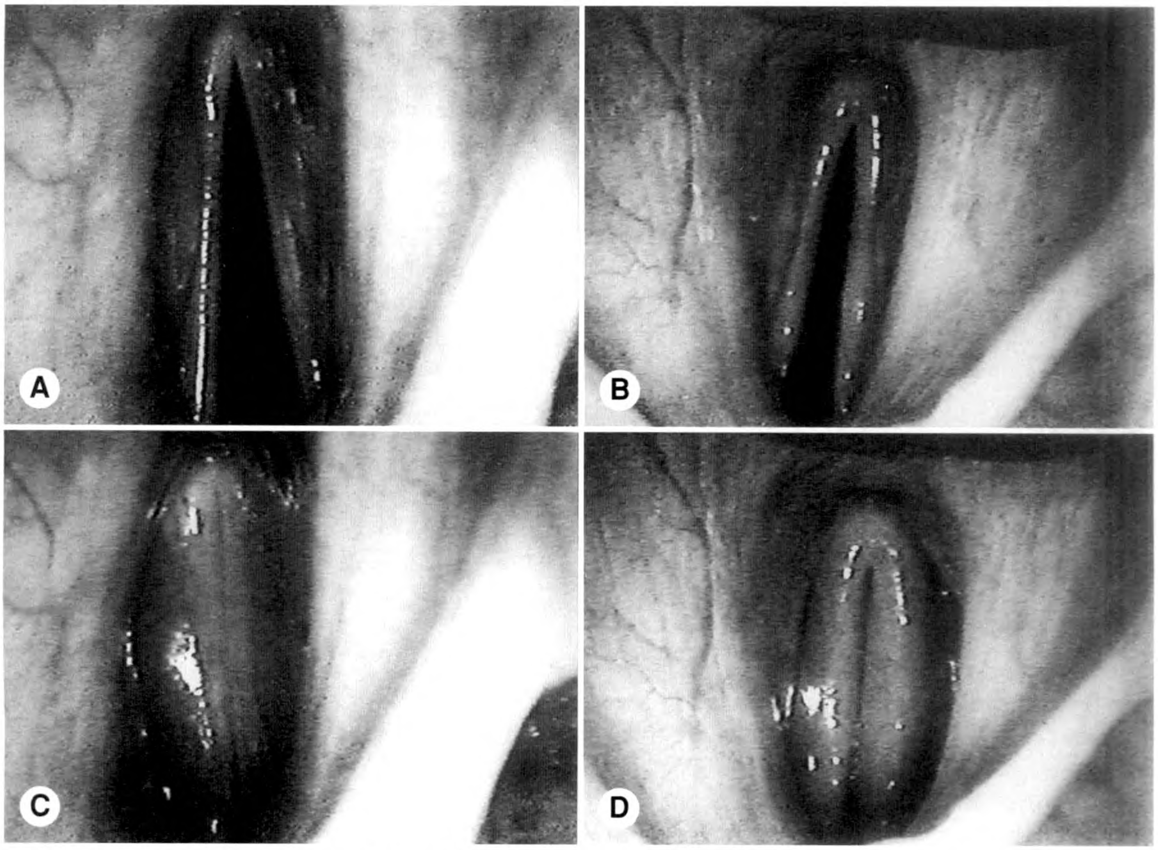

Fig 2. Vocal fold appearance. A) Control, RLN stimulation off. B) Treatment, RLN stimulation off. C) Control, RLN stimulation on. D) Treatment, RLN stimulation on.

.23, unpaired *t*-test).

Three of the 8 control animals and 5 of the 8 experimental animals were judged to be injected ($p = .62$, 2-tailed Fisher's exact test). The appropriate side of vocal fold injection was identified in 3 of the 8 experimental animals. Of the 48 individual judgments (3 reviewers × 16 animals) regarding classification into control and injected groups, without regard to identification of the injected side, 26 or 54% were correct. There were 13 of 24 (54%) correct assessments regarding injection in both the control and experimental groups. The ability of the reviewers to determine a history of vocal fold injection was not significantly different from random.

DISCUSSION

Doxorubicin is an anthracycline antibiotic produced by the fungus *Streptomyces peucetius*. It is an effective chemotherapeutic agent against carcinoma of the breast, ovary, and lung, as well as lymphomas and sarcomas. Shortly after intravenous administration, most of the drug is bound to DNA, from which it is slowly released. The liver is the major site of drug clearance, where the side chain ketone is reduced to an alcohol.[7] One of the primary mechanisms by which doxorubicin kills cells is by insertion between successive DNA base pairs, inhibiting nucleic acid synthesis and mitotic activity.[7] Metabolism of doxorubicin leads to production of free radicals, which damage cell membranes by lipid peroxidation.

The clinical usefulness of doxorubicin as a chemotherapeutic agent is limited by the development of a dose-dependent cardiomyopathy. Anthracycline cardiomyopathy is characterized by dilation of sarcoplasmic reticulum and loss of myofibrils. Damage to the sarcoplasmic reticulum leads to defective calcium binding. The result is an impaired muscle relaxation and an elevated level of free calcium. Proteases activated by elevated calcium levels cause muscle fiber loss and decreased myocardial activity. Free radical formation also plays an important role in the cardiotoxicity of doxorubicin. Other mechanisms, such as a direct effect on adenosine triphosphate hydrolyzing enzyme (ATPase) activity of cellular fragments and on the calcium release channels of the sarcoplasmic reticulum, have also been hypothesized.

Cardiac muscle is probably not a specific target of doxorubicin-induced toxicity. The amount of damage to a muscle cell is directly related to the local concentration of the drug.[8] After intravenous or intraperitoneal injection, the concentration of doxorubicin in skeletal muscle is approximately one eighth the concentration seen in cardiac muscle.[8] It is not surprising that skeletal muscle is spared relative to cardiac muscle during intravenous doxorubicin treatment for malignancy. Exposure of skeletal muscle to doxorubicin in amounts equivalent to those seen in the heart produces myopathic changes similar to those seen in cardiac muscle.[8]

The specific myotoxic effects of doxorubicin have potential in the treatment of dystonia. Local injection is necessary for doxorubicin to benefit patients with dystonia. Extravasation of doxorubicin at doses intended for systemic use results in pain, erythema, and swelling. Within a few weeks, ulcer formation and necrosis of deep subcutaneous structures can occur. Intradermal injection of doxorubicin produces a dose-dependent, local toxicity. A full-thickness cutaneous ulcer reliably develops after injection of 1.0 mg of doxorubicin in the porcine flank.[9] Within an hour after injection, most of the doxorubicin is bound to tissue sites, and attempts at reversal of toxicity by inactivation of doxorubicin are ineffective.[9]

The recommended treatment of doxorubicin extravasation is early excision of the affected tissues. Continuing necrosis of surrounding noninfiltrated tissue can occur when surgical excision is delayed.[10] This process is thought to be caused by the release of doxorubicin following cell death. The now-free doxorubicin can be taken up by nearby cells, with the process repeating itself.[7] Doxorubicin has been demonstrated at the injection site 28 days after extravasation.[11]

Two months was felt to be adequate to study the toxic effects of doxorubicin. We reasoned that if excessive toxicity occurred, then further investigation of doxorubicin was probably not warranted. If, however, the acute toxicity was tolerable and undesirable side effects were limited, then we would conclude that further research should be encouraged. In studies on intradermal injection in the porcine model, maximal ulceration was present at 3 weeks, with complete healing at 7 weeks.[9] In a study of rats, fluorescence (indicating presence of doxorubicin) was no longer present by day 27 after injection.[12] In an acute histologic study of the rabbit eyelid, muscle kill had been accomplished after 4 days.[13] In another study, examining the quantitative muscle loss after injection of doxorubicin into monkey eyelids, no difference in muscle loss was seen between 2 and 5 months.[14]

The acute toxicity associated with peripheral doxorubicin injection was minimal in this study. In a pilot study, no vocal fold swelling was observed on direct laryngoscopy 48 hours after vocal fold injection with doxorubicin (up to 8 mg) and verapamil (unpublished observations). Verapamil was used in this study because of its ability to increase the effective muscle toxicity of doxorubicin.[15] A reduced dose of doxorubicin is able to be injected while continuing to produce an effective myectomy. Verapamil is a calcium channel blocker that inhibits influx of calcium through slow channels into muscle cells. Intracellular accumulation of doxorubicin is increased when it is given along with verapamil. The increased intracellular concentration of doxorubicin probably accounts for at least some of the increase in muscle toxicity. Verapamil alone, however, was found to have a permanent myotoxic effect when injected into the eyelid.[15] A direct toxic effect of verapamil may contribute to the increased effectiveness seen when it is combined with doxorubicin.

The chemomyectomy occurring after doxorubicin injection is accompanied by toxic effects on the associated motor nerve. Doxorubicin injected systemically does not penetrate the blood-brain barrier.[16] Localized central nervous system damage can occur, however, after intraneural or intramuscular injection.[16-18] Motor fiber degeneration in rats can occur as early as 6 days after injection,[17] with progressive degeneration occurring as late as 39 days.[18] Retrograde transport of doxorubicin after intramuscular injection into the gastrocnemius muscle of rats resulted in destruction of motor neurons in the anterior horn of the spinal cord, while sparing other central neurons.[17] Degeneration of hypoglossal motor neurons has also been demonstrated after doxorubicin injection into the tongue.[19] Motor neurons are more susceptible to the toxic effects of intraneural injection of doxorubicin than are sensory neurons.[18] With appropriate dosing, a selective motor degeneration can occur.[18] The additional effect of selective chemodenervation of the injected muscle could reduce the potential of symptom return after treatment of a focal dystonia.

Local injection of doxorubicin in the eyelid is tolerated better than injection in other cutaneous sites.[20] The increased blood supply present in the head and neck region and in the underlying striated muscle was felt to be responsible for the increased tissue tolerance. Marked atrophy of the orbicularis oculi muscle was evident 21 days after doxorubicin injection, without disruption of the overlying epithelium.[20] Ten weeks after a 2-mg injection of doxorubicin into the eyelid, a selective muscle injury was observed. Very few muscle fibers remained, despite a relatively normal appearance of the preseptal region.[21] Chemomyectomy of the orbicularis oculi muscle resulted in eyelid weakness lasting greater than 2.5 years in 6 of 8 patients with hemifacial spasm.[22] Repeated doxorubicin injections of 0.8 to 1.5 mg, with a cumulative dose of 0.8 to 7.0 mg, were used. Doxorubicin injection was noted to cause loss of subcutaneous fat and scarring of the eyelid tissue. In most patients this was felt to be aesthetically pleasing and similar to a "chemical" blepharoplasty.

The functional requirements of the vocal fold are different from those of the eyelid. For clear voice production, the epithelium must be able to vibrate in a wavelike fashion. Disruption of the fluid properties of Reinke's layer or extensive fibrosis of the underlying muscle adversely affects the mucosal wave and the resultant vocal quality. It is critical that any treatment intended to improve voice not disturb the mucosal wave. To be an effective alternative treatment for laryngeal dystonia, doxorubicin should weaken the thyroarytenoid muscle without interfering with the mucosal wave.

In the present study, unilateral injection of 3 mg of doxorubicin and 0.5 mg of verapamil into the canine vocal fold reliably reduced the evoked vocal fold tension without evidence of airway complications. Differences in vocal fold appearance and the ability to develop a mucosal wave between injected and noninjected vocal folds in this model were minor and not obvious by videostroboscopy 2 months after injection.

The present study does *not* provide justification for clinical trials evaluating doxorubicin in the treatment of laryngeal dystonia. A number of concerns remain. These include the possibility of an additional muscle toxicity over a longer postinjection period, a lack of data on combined treatment with botulinum toxin, and the potential for additional central nervous system toxicity. Formal long-term carcinogenicity studies on doxorubicin are lacking, but doxorubicin does have mutagenic properties. The results of this study, however, support further laboratory investigation of chemomyectomy as a potential alternative treatment for laryngeal dystonia.

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RARE BENIGN TUMORS: LARYNGEAL AND HYPOPHARYNGEAL LIPOMATA

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Lipomata of the larynx and the hypopharynx are very rare benign lesions; as they look macroscopically like retention cysts, their diagnosis is usually made after surgery. Sometimes, lipomata of the hypopharynx become very large and life-threatening. Plain fluoroscopy and fluoroscopy aided with barium often fail to depict the lesion if it is localized subepiglottically; standard barium swallow examination and computed tomography allow an accurate diagnosis to be made in many cases, although magnetic resonance imaging is still more accurate and allows not only a more specific diagnosis of the lesion, but also a better depiction of the origin of the frequently pedunculated tumor and its extension into the parapharyngeal space. This paper adds 6 cases of hypopharyngeal and 1 case of laryngeal lipoma to the literature and discusses modern diagnostic and therapeutic strategies.

KEY WORDS — carbon dioxide laser resection, hypopharyngeal lipoma, laryngeal lipoma, magnetic resonance imaging, suspension laryngoscopy, ultrasound.

INTRODUCTION

Lipoma of the hypopharynx is rare and usually arises in the aryepiglottic fold. In 1952, Som and Wolff[1] reported 33 such tumors and added 2 of their own cases. In their literature review, Laurent et al[2] reported 19 additional cases with solitary hypopharyngeal tumors and 6 cases with multiple tumors and added 2 of their own cases.

In general, these benign neoplasms are encapsulated, smooth, and usually pedunculated. Symptoms are few and uncharacteristic, and preoperative diagnosis is difficult. Clinically, they can be confused with other benign lesions, such as retention cysts or laryngoceles. Even if performed, native fluoroscopy and barium flow fluoroscopy often fail to detect the lesion.[2] Considerable improvement in the radiologic assessment of the lesion has been reported for standard barium swallow examination and computed tomography (CT),[3,4] although compared to CT, magnetic resonance imaging (MRI) diagnosis possesses some major advantages: 1) its multiplanar imaging capability allows one to obtain coronal and sagittal images to define more accurately the origin of the peduncle; 2) its superior soft tissue contrast improves differentiation and depicts the lesion's extension into the prelaryngeal or parapharyngeal space as well as its relationship to other cervical structures; 3) the patient is not exposed to ionizing radiation, and there is no need for iodine contrast agents; and 4) the appearance of the hypopharyngeal lipoma is characteristic and unequivocal and permits uneventful resection with a suspension laryngoscope and carbon dioxide (CO2) laser.

During the 3- to 8-year follow-up (mean 4.2 years) of our 7 patients, no lipoma recurrence was noted.

PATIENTS AND METHODS

Between 1990 and 1997, 6 patients received a diagnosis of hypopharyngeal lipoma, and 1 patient, of laryngeal lipoma, at the otolaryngological outpatient unit of the University of Cologne. Whereas 6 of them did not experience an emergency situation, patient 5 arrived during the night at the emergency unit (Fig 1). The leading symptom in the 6 patients with hypopharyngeal lipoma was difficulty in swallowing (see Table). The patients with hypopharyngeal lipoma complained of intermittent symptoms. However, the patient with laryngeal lipoma, a 46-year-old woman (patient 7), showed different symptoms; she suffered from hoarseness, cough, and stridor, and there were no symptom-free intervals. Moreover, her lipoma was much smaller at diagnosis than the hypopharyngeal lipomata (Fig 2).

Two patients (Nos. 3 and 4) had undergone surgery 17 and 27 and 16 years earlier, respectively, for

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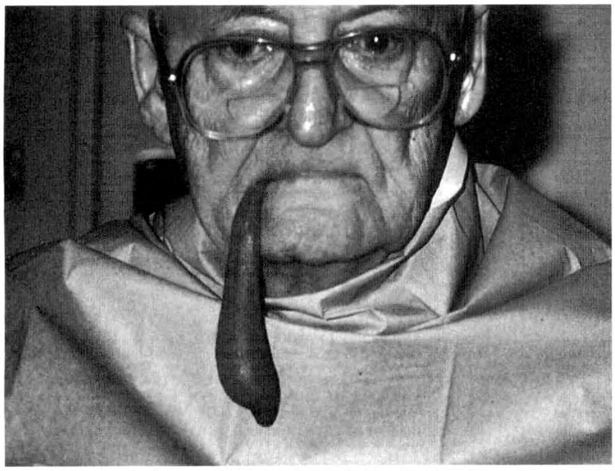

Fig 1. (Patient 5) Eighty-three-year-old patient presenting after having vomited tumor with length of 15 cm measured from teeth. Histopathologic examination revealed fibrolipoma.

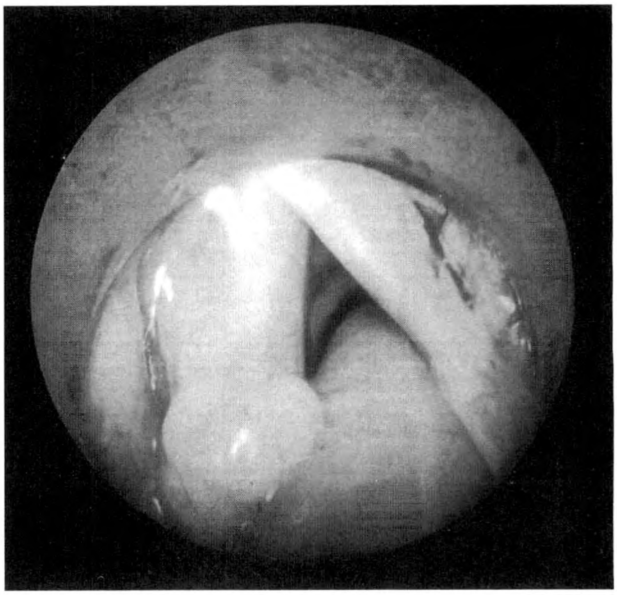

Fig 2. (Patient 7) Suspension laryngoscopy showing small laryngeal lipoma emerging from left false vocal fold of 46-year-old patient.

laryngeal "polyps." Records were not available for these procedures. During diagnostic workup, we performed ultrasound and MRI of the neck region on 5 of the 7 patients, performed standard barium swallow examination on 2 patients, and performed CT on 3 patients.

RESULTS

Patient 5. The patient's medical history had been without complaints. Suddenly suffering from nausea due to gastroenteritis, he vomited a long, pedunculated tumor with a yellowish surface that he tried in vain to remove forcibly. Laryngoscopy revealed the peduncle of the lesion emerging from close to the left arytenoid cartilage. The fibrolipoma was removed immediately during suspension laryngoscopy by use of CO_2 laser equipment. There has been no recurrence of the disease for 5 years.

Ultrasound. Five of the 7 patients who were referred to our outpatient clinic underwent preoperative ultrasound investigative screening of the neck region (Sector scanner, 7.5 MHz, Toshiba Sonolayer V Sal 38B or Philips Sonodiagnost 360 [since 1994]). None of them were found to have cervical disorders. Neither the pedunculated lipomata, situated behind the thyroid cartilage and predominantly surrounded by air, nor their peduncles were depicted by ultrasound.

CLINICAL FEATURES OF LARYNGEAL AND HYPOPHARYNGEAL LIPOMATA

Patient	*Age at Diagnosis*	*Sex*	*Complaints*		*Duration of Symptoms*	*Insertion of Lipoma*	*Histopathology*	*Follow-up (y)*	*Previous Surgery*
			Intermittent	*Constant*					
1	45	M	Difficulty in swallowing		>1 y	Left aryepiglottic fold	Lipoma	8	
2	76	M	Cough, stridor		1 y	Right pyriform recess	Lipoma	8	
3	63	F	Difficulty in swallowing		>1 y	Left aryepiglottic fold	Lipoma	4	1980
4	72	M	Difficulty in swallowing, cough		1 y	Left aryepiglottic fold	Lipoma	7	1970, 1981
5	83	M	*		5 h	Left arytenoid cartilage	Fibrolipoma	5	
6	57	M	Cough, stridor, hoarseness		>1 y	Right pyriform recess	Lipoma	4	
7	46	F		Difficulty in swallowing, cough, hoarseness	<1 y	Left false vocal fold	Lipoma	3	

*See Patients and Methods.

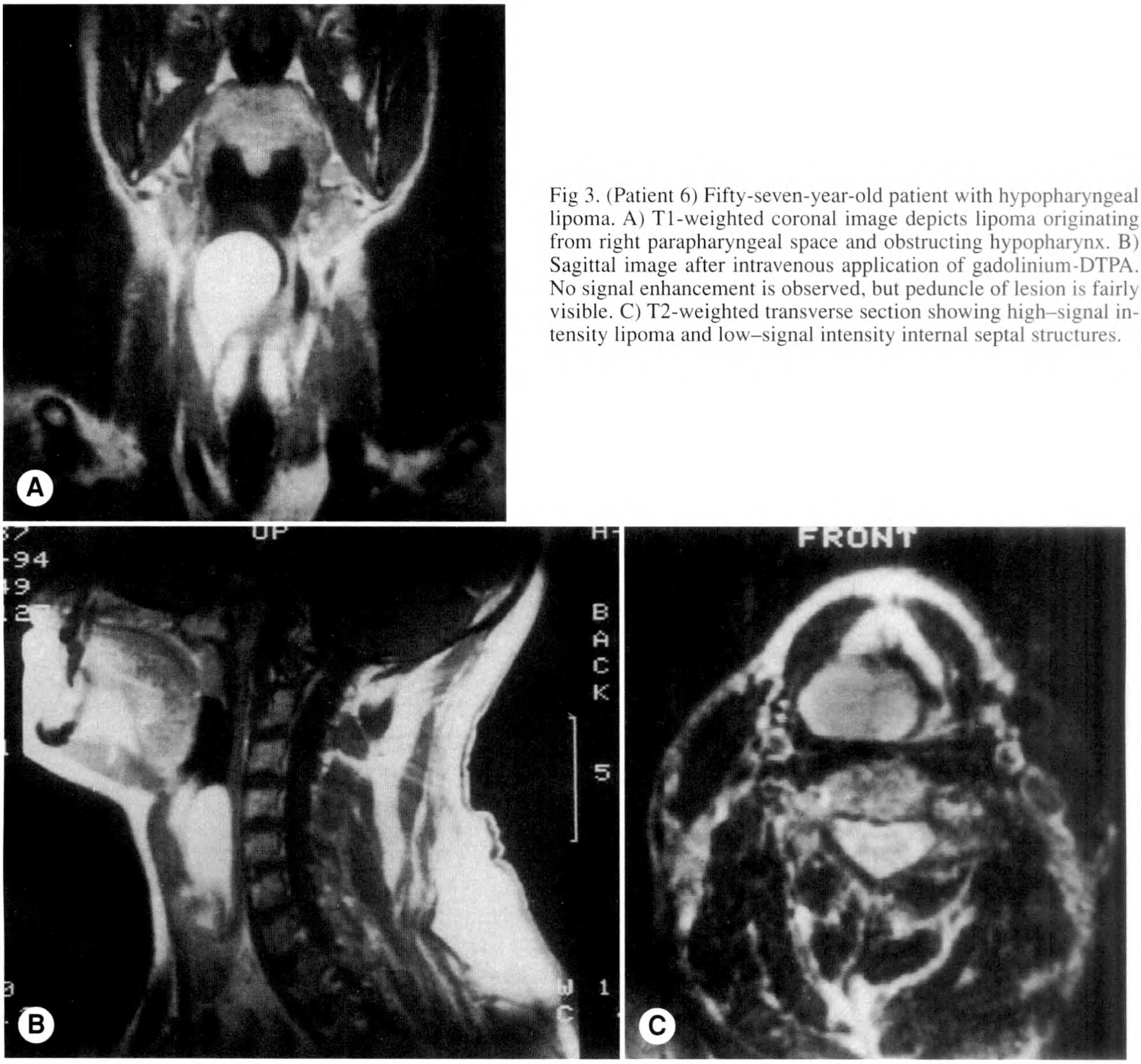

Fig 3. (Patient 6) Fifty-seven-year-old patient with hypopharyngeal lipoma. A) T1-weighted coronal image depicts lipoma originating from right parapharyngeal space and obstructing hypopharynx. B) Sagittal image after intravenous application of gadolinium-DTPA. No signal enhancement is observed, but peduncle of lesion is fairly visible. C) T2-weighted transverse section showing high–signal intensity lipoma and low–signal intensity internal septal structures.

Standard Barium Swallow Examination. We performed standard barium swallow examination on 2 of our patients (Nos. 1 and 6) suffering from hypopharyngeal lipoma during the diagnostic workup. The barium swallow study revealed a marked lateral and posterior deviation of the contrast column caused by the mass in both patients. The lesions were diagnosed as being large, with smooth borders, and were located in the hypopharynx. No information was obtained about the origin of the peduncle or the tissue characteristics of the tumor, however.

Computed Tomography. During the diagnostic workup, we performed contrast-enhanced CT scans on 3 patients. Computed tomography showed the lipomata to be typically homogeneous with a low attenuation value and a density lower than water (<0 Hounsfield units), and accurately revealed the extent of the lesions. However, CT was not able to define the origin of the peduncles due to its inability to obtain strictly coronal or sagittal sections of the neck and its low spatial resolution of coronal and sagittal formations.

Magnetic Resonance Imaging. We performed preoperative MRI on 5 patients using a 1.5-T MRI system and a head and neck coil. Transverse sections of the neck, with slice thicknesses of 4 mm and fields of view of 230 mm, were obtained. We acquired T1-weighted (TR = 500 milliseconds [ms], TE = 27 to 30 ms) and T2-weighted (TR = 2,000 ms, TE = 80 ms) spin echo images before, and T1-weighted images after intravenous application of gadolinium-diethylenetriamine pentaacetic acid (DTPA). The signal intensities of the lipomata in the T1- and T2-weighted sequences were similar to those of subcutaneous fat, thus suggesting a fatty lesion (Fig 3A). No enhancement after the application of gadolinium-DTPA was observed — a finding suggesting a be-

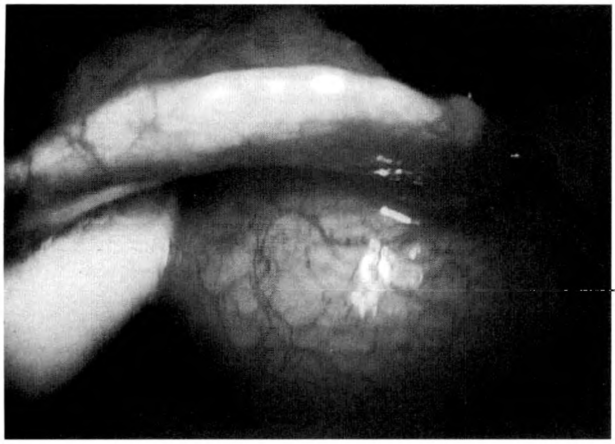

Fig 4. (Patient 1) Suspension laryngoscopy before carbon dioxide laser resection. Glottic plane is not visible.

nign lesion (Fig 3B). Additional T2-weighted (TR = 1,500 ms, TE approximately 100 ms) sections were obtained to characterize the tissue more exactly.

Moreover, the MRI detected some septal structures of lower signal intensity subdividing the high–signal intensity lesion into compartments, thus suggesting the diagnosis of a lipoma (Fig 3C). Coronal and sagittal MRI slices revealed the tumor's peduncle, in most cases originating from parapharyngeal fat and in 1 case from prelaryngeal fat (Fig 3A,B). In all 5 patients, MRI allowed an exact diagnosis of tumor localization, the insertion of its peduncle, and its lipomatous character.

Suspension Laryngoscopy and Follow-up. Suspension laryngoscopy with microscopic magnification detected the tumor in all patients, although it proved difficult to explore the lower portion of the tumor in 4 patients (Nos. 1, 2, 4, and 6) because of the narrowing of the pyriform fossa and the extent of the smooth tumor. Due to the size of the tumor, the glottic plane could not be seen in 2 patients (Nos. 1 and 2; Fig 4). In patients 3 and 5, the peduncle was clearly visible during laryngoscopy. Using a CO_2 laser, we removed all tumors in one piece endoscopically. The resected tumors measured between 1 cm (patient 7) and 23 cm (patient 5) in diameter and macroscopically appeared yellowish on the cut surface (Fig 5). A tracheostomy was not necessary in any of the patients, and nasogastric feeding tubes were removed on the fifth postoperative day. Recovery was uneventful in all cases. Histologic examination demonstrated adult fat cells and fine fibrous septa covered by a layer of normal stratified squamous epithelium in all the examined lipomata. A 36-month follow-up, which included laryngoscopy, did not reveal any recurrence (last laryngoscopy, December 1997); the 2 patients operated on before (Nos. 3 and 4) are without recurrence as well.

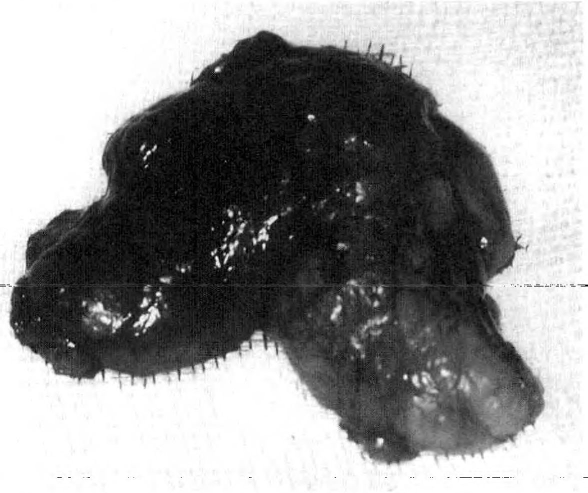

Fig 5. Typical resection specimen showing cut surface.

DISCUSSION

Neoplasms derived from fatty tissue are distinctly rare in the hypopharynx, and even more infrequent in the larynx.[5] The location and size of the tumor determine whether it will be discovered and the types of symptoms a patient will have. Small lipomata, or even those of moderate size, may produce no symptoms and may only be discovered accidentally on routine inspection of the larynx and pharynx,[5] as in patient 1. Dysphagia, discomfort, and an irritating or paroxysmal cough are the predominant symptoms of the more bulky tumors. Five of the 6 patients suffering from hypopharyngeal lipoma complained of intermittent symptoms, probably due to the temporary displacement of the pedunculated lesion. Patient 5 had never had any symptoms until his queasiness caused vomiting for the first time in 15 years. It has to be assumed that his 23-cm lipoma was suspended from the cervical part of the esophagus, as described by Mansson et al[6] in 1974.

The most serious complication of pedunculated hypopharyngeal tumors is sudden laryngeal obstruction. This complication may cause hoarseness or stridor, and occasionally can result in asphyxiation and death.[7-9]

The so-called lipomata, which occur more often in men, are actually, in most instances, fibrolipomata containing a meshwork of strands of fibrous tissue. There has been no report of malignant transformation of solitary lipoma, whereas malignant change in multiple lipomata of the larynx and pharynx has been described.[1,10] Jesberg[11] points out that clinical malignancy of histologically benign lesions, though formerly suspected, is not a general feature of lipoma and fibrolipoma, confirming our own observations.

In our group of patients, MRI was the most helpful presurgical imaging tool. Ultrasound did not depict the benign lesion, perhaps because of cartilage shadowing and surrounding air, in 5 patients.

A standard barium swallow examination depicted the hypopharyngeal lesion in 2 patients. However, no information was obtained about the tissue characteristics or peduncle origin of the lesion. From our point of view, this method does not add any important diagnostic information to the endoscopically obtained impression. There is no tissue definition, subepiglottic lesions are missed, and the radiation exposure of the thyroid gland is rather high.

On CT scans, fat tissue shows up as typically homogeneous, with a low attenuation value and a density lower than that of water (<0 Hounsfield units). Therefore, CT not only reveals the extent of the tumor, but also establishes its lipomatous nature with a high degree of confidence.[3]

Compared to CT, MRI consistently shows superior soft tissue definition, and its ability to produce direct coronal and sagittal images allows the visualization of intrinsic laryngeal musculature in addition to better definition of the cranial-caudal tumor extension. In our study, lipomatous tissues were sharply defined, and peduncles of hypopharyngeal lipomata were clearly depicted in 3 of 5 patients.

Another advantage of MRI is that the patient is not exposed to ionizing radiation and there is no need for iodine contrast agents. Moreover, the previously mentioned motion artifacts[12] have disappeared, since MRI measurement times have diminished considerably (approximately 15 minutes for the acquisition of T1- and T2-weighted images).

Endoscopically, lipomata are sometimes difficult to differentiate from other benign lesions, such as retention cysts or laryngoceles, or even malignant lesions, such as liposarcoma.[13] In these cases, MRI achieves a considerable improvement in the preoperative diagnosis.

Especially in the 4 patients (Nos. 1, 2, 4, and 6) whose tumor size prevented laryngoscopic visualization of the glottic plane and the pyriform recesses, MRI was of great practical value and allowed exact presurgical assessment of the extent and nature of the tumors, thus enabling adequate surgical treatment.

CONCLUSION

Benign tumors of the larynx and hypopharynx, such as lipomata, are rare, and differential diagnosis is difficult. For choosing an appropriate therapeutic strategy, accurate imaging techniques are necessary, particularly if the size of the lesion makes endoscopic evaluation difficult. Whereas ultrasound does not appear to be valuable in this rare situation, MRI improves and facilitates the diagnosis of hypopharyngeal lipoma and its anatomic relationship to other structures of the neck. Transoral resection of the lipoma is relatively simple and effective with use of a CO_2 laser. Although described in the literature, recurrence of the disease seems to be rare.

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SCLEROMA OF THE LARYNX AND ASSOCIATED SQUAMOUS CELL CARCINOMA: A CAUSAL RELATIONSHIP?

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The known risk factors for development of laryngeal carcinoma are smoking, alcohol drinking, air pollution, laryngeal keratosis, single adult papillomas, and previous irradiation. Laryngeal scleroma as a risk factor for the development of laryngeal carcinoma has not been mentioned in the English-language literature, to my knowledge. This is a report of 2 cases of proven long-term rhinolaryngoscleroma that have changed to laryngeal carcinoma.

KEY WORDS — cancer, larynx, scleroma.

INTRODUCTION

The majority of neoplasms arising in the larynx are squamous cell carcinoma. It constitutes 1% of all cancers among males and approximately 0.1% among females.[1] The laryngeal cancer peak incidence is at age 60 to 62 years. It is due to a variety of known environmental irritants. The relationship between laryngeal cancer and smoking has been known for many years.[2] The incidence of cancer of the larynx increases with alcohol consumption, particularly in the supraglottic larynx. Industrial exposure has been considered as a cause of laryngeal cancer in a variety of substances, such as asbestos,[3] mustard gas,[4] wood,[5] and nickel.[6] Two cases of radiation-induced squamous cell carcinoma have been reported.[7] Decreased intake of vitamins A and C doubles the risk of laryngeal carcinoma.[8] It is known that vitamin A deficiency causes epithelial metaplasia.[9] Maran et al[10] reported that there is a high prevalence of postcricoid carcinoma in Scandinavian countries that might be due to a deficiency of ascorbic acid with secondary reduced iron absorption. There is a 4.3% chance of laryngeal keratosis' changing to invasive carcinoma.[11] Between 2% and 3% of solitary laryngeal papillomas contain malignant neoplasms.[12]

Scleroma was first described by Von Hebra in 1870 as a nasal disease — hence the name rhinoscleroma.[13] Gerber in 1900 characterized the lesion as a chronic inflammatory process accompanied by perivascular cellular infiltration and connective tissue proliferation.[14] It is a progressive granulomatous disease that begins usually in the nose and may extend to involve the nasopharynx, oropharynx, larynx, and sometimes the trachea and bronchi.[15] In 1876, Gerhard gave the first comprehensive description of laryngoscleroma.[16] Laryngotracheal manifestations of rhinoscleroma are uncommon, and they are potentially life-threatening because of progressive airway obstruction.[17] The incidence of laryngotracheal scleroma ranges from 15% to 80%.[18,19] Isolated laryngotracheal disease without nasal involvement has also been reported.[20]

Scleroma may affect persons of either sex and at any age. It may be seen anywhere in the world, and people of any race may be affected. It is endemic in countries that have a poor standard of domestic hygiene, such as Pakistan, Indonesia, and Egypt.

The majority of scleroma patients present with nasal symptoms such as nasal obstruction, fetid rhinorrhea, nasal crusting, loss of smell, and/or partner complaints of fetor oris. It is uncommon for the scleroma patient to present with dry cough, mild hoarseness, and mild stridor. However, scleroma is easily diagnosed clinically in endemic areas.

The causative organism is the gram-negative Frisch bacillus (*Klebsiella rhinoscleromatis*). Toppozada et al[21] found a high titer of antibodies in the serum of patients with rhinoscleroma. Histologically, granulomatous tissue infiltrates the submucosa and is characterized by the presence of plasma cells, lymphocytes, and eosinophils among which are scattered large foam cells (Mikulicz cells) that have a central nucleus and a vacuolated cytoplasm containing Frisch bacilli and Russell bodies resembling plasma cells and having an eccentric nucleus and deep eosin-staining cytoplasm.

Scleroma arises at a junction line of 2 types of epithelium. Clinically, there are 3 stages: the atrophic stage, characterized by mucosal changes and crust formation; the granulomatous stage, with small, firm, granulomatous nodules anywhere in the nose, pharynx, larynx, or bronchi; and the fibrotic stage, in which dense fibrotic narrowing take place.[22]

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Spread to lymph nodes has been reported, but it is extremely uncommon and is thought to be prevented by early fibrous tissue deposition blocking the lymphatics.[23] Bone may be extensively involved.[24]

There are a variety of treatments in the form of systemic and local antibiotics, local acriflavine, radiotherapy, autogenous vaccine, electrical cauterization, cryocauterization, fever therapy, surgery, and carbon dioxide surgical laser.[25]

Clinical remission and relapse characterize the disease, making diagnosis and treatment difficult. This is because the organism remains dormant in an anaerobic spore form and is reactivated months to years after proper antibiotic treatment, when, I think, the immunity of the patient decreases.

In 1956, Kouw Ennar[26] noted that changing of scleroma to malignancy is rare, and stated that actual carcinomatous degeneration occurs in 5% of rhinoscleroma patients (cited by Singh and Srivastava[27]). Yassin and Safwat[16] in 1966 reported a case of rhinopharyngoscleroma that changed to a malignant palatal lesion. Singh and Srivastava[27] in 1972 reported another case of a malignant palatal lesion after a period of rhinopharyngoscleroma. As far as I know, no one has reported a case of laryngoscleroma converting to laryngocarcinoma in the English-language literature. This is a report of 2 cases of rhinolaryngoscleroma, changing to subglottic and supraglottic carcinoma in 1 case and glottic carcinoma in the second case.

CASE REPORTS

Case 1. A 65-year-old man presented to our department with mild-to-moderate stridor. After his admission, the relevant medical history included that 10 years earlier he had been examined in the outpatient clinic of our professor, at which time a biopsy proved rhinoscleroma. He had been given streptomycin injections 1 g daily for 40 days. He had not been seen since that time, ie, for 10 years. On examination, his nose showed greenish crusts, mucopurulent discharge, and multiple small granulomata covering and obscuring both inferior turbinates, his mouth showed an intact mucosal lining, the oropharynx showed fusion of the soft palate and posterior pillars with the posterior pharyngeal wall, leaving a small slitlike aperture in the midline, the uvula was absorbed, and its base was fibrosed (uvula sign). Fiberscopic examination showed a subglottic mass that circumferentially obstructed 70% to 75% of the subglottic area, which I thought was a scleromatous mass. At night, the patient developed severe stridor, and a tracheostomy was performed. The following day, the patient was taken to the operating room, where direct laryngoscopy was performed, revealing a huge mass involving both the subglottic and supraglottic regions and obstructing 70% to 75% of the lumen. Separate biopsy specimens were taken from the subglottic and supraglottic regions. Histopathologic examination of both specimens revealed evidence of focal surface ulceration associated with involvement by an invasive malignant squamous epithelial growth displaying moderate overall cellular anaplasia with mild-to-moderate associated keratinization. Focal tumor devitalization was seen, as well as reactive, nonspecific inflammation and stromal fibrosis (Fig 1A). Another biopsy specimen was taken from the granulomatous lesion of the nasal cavity and proved to be scleroma (Fig 1B). Surprisingly, the patient had no lymph node enlargement at the neck.

Total laryngectomy was performed, and the removed larynx was examined, showing a huge, whitish, firm mass infiltrating both subglottic and supraglottic regions (Fig 1C). The patient has a permanent tracheostomy and is under follow-up and medical treatment for scleroma.

Case 2. A 90-year-old man had presented 3 years earlier to the outpatient clinic of the same professor with a history of dysphonia that was diagnosed as chronic laryngitis and was relieved under medical treatment. One year later, he presented to the same outpatient clinic with the same complaint. Clinically, his nose revealed an unhealthy atrophic mucosal lining with areas of greenish crusts involving the middle meatus, the inferior turbinate, and the meatus of both sides. His pharynx was free except for some crusts that were covering the posterior pharyngeal wall. Laryngeal examination revealed a subglottic reddish mass circumferentially involving the region and obscuring 50% of the subglottic space, with some crusts. A biopsy was taken from the nasal mucosa and yielded a diagnosis of rhinopharyngoscleroma on histopathologic examination. Medical treatment of scleroma was given in the form of Rimactane (rifampin) capsules 300 mg twice daily before breakfast and dinner for 40 days plus vitamin A pills. The patient had no follow-up for 2 years. The patient returned after 2 years of medical treatment with a history of dysphonia and mild stridor. Examination revealed an unhealthy nasal mucosa without any crusts and a normal pharynx, and indirect laryngoscopy revealed a left vocal fold mass with no crusts.

Postcontrast computed tomographic examination of the larynx and neck revealed an ill-defined small soft tissue lesion involving the left laryngeal ventricle and the left vocal fold. Associated subtle thickening of the left aryepiglottic fold was noted. The anterior and posterior commissures showed no obvious gross infiltration. The laryngeal skeleton, pre-

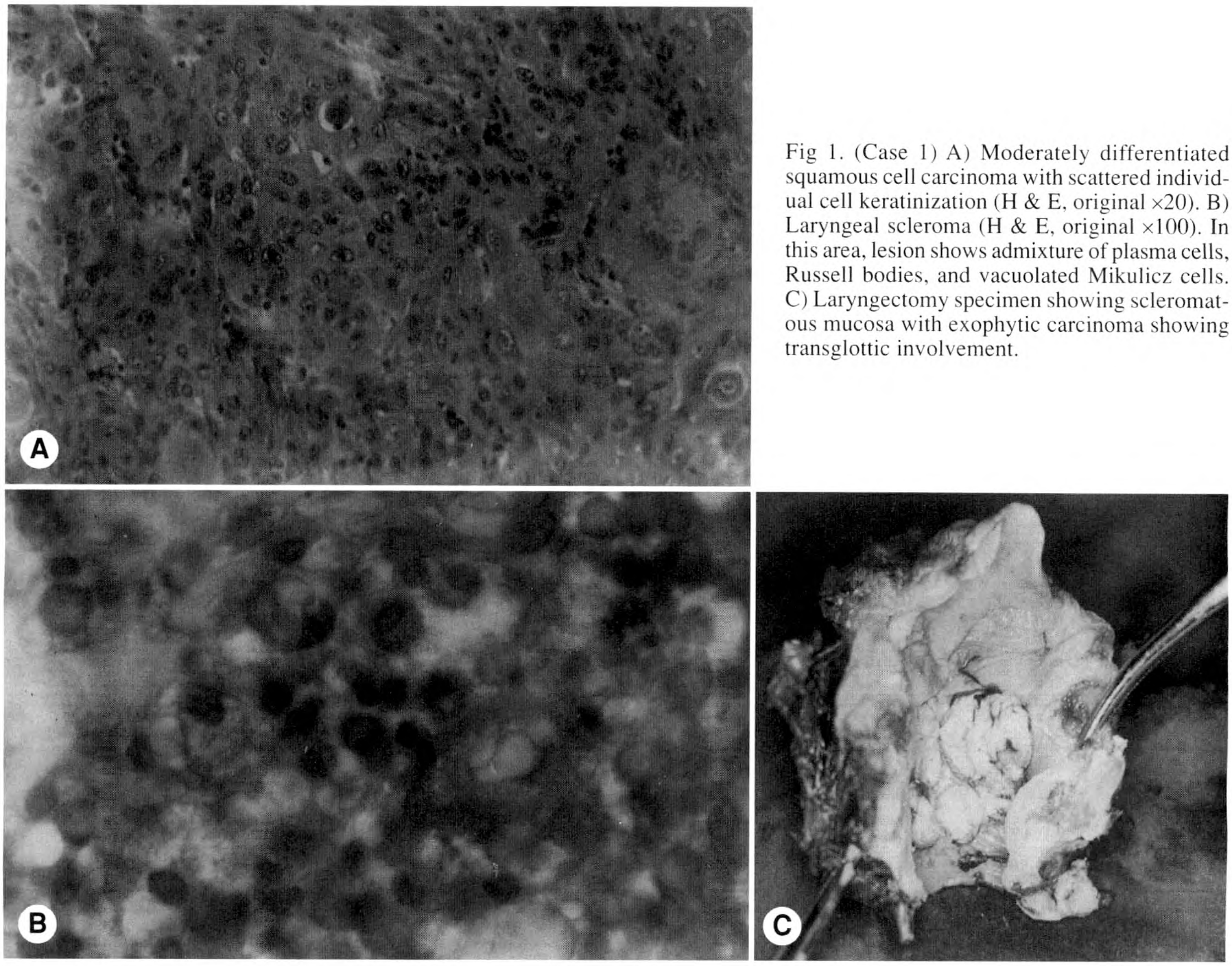

Fig 1. (Case 1) A) Moderately differentiated squamous cell carcinoma with scattered individual cell keratinization (H & E, original ×20). B) Laryngeal scleroma (H & E, original ×100). In this area, lesion shows admixture of plasma cells, Russell bodies, and vacuolated Mikulicz cells. C) Laryngectomy specimen showing scleromatous mucosa with exophytic carcinoma showing transglottic involvement.

epiglottic space, valleculae, and pyriform fossae were intact. No infraglottic lesions were seen. No extralaryngeal infiltrations were seen. No pathologically enlarged cervical lymph nodes were seen. The patient was admitted to our university hospital. Direct laryngoscopy was performed and a biopsy specimen was taken from this mass.

Histologic examination revealed a malignant squamous epithelial growth formed of squamoid nests and moderate keratin formation. The neoplastic cells exhibited moderate anaplastic cellular changes associated with a relatively elevated mitotic index. The intervening stroma was fibrocollagen and showed a scattered, mild, nonspecific inflammatory cell infiltrate. Foci of tumor necrosis were also seen. A histopathologic diagnosis of invasive squamous cell carcinoma grade 2 was reported (Fig 2).

The patient was then referred to a radiotherapist. He has been receiving a course of 64 Gy/wk for 6.5 weeks.

DISCUSSION

It is very rare to find a case of scleroma becoming malignant. These 2 cases of laryngeal scleroma becoming laryngocarcinoma may be the only reported cases in the English-language literature. Two cases of rhinoscleroma becoming palatal carcinoma were reported in 2 papers.[16,27] There is increasing recognition that the development of malignancy in laryngeal mucosa is a process requiring several years' duration and that it takes place in a stepwise manner very similar to that demonstrated in bronchial epithelium. When subjected to an irritating substance (cigarette smoke) or mechanical trauma (voice abuse), laryngeal mucosa reacts by transforming itself into a tougher mucosa (squamous metaplasia) by increasing the mucosal thickness (acanthosis or squamous metaplasia) or by developing a keratin layer of the surface (keratosis).[1] A possible explanation of the etiopathogenesis of the malignant change of scleroma is that the organism and the specific degenerative cells and repeated trauma by crusts act as irritant substances, similar to bilharzial ova in the development of bladder carcinoma. Although the exact cause of malignant transformation in bilharzial carcinoma in the bladder is unknown, the suspected factor is the repeated trauma produced by penetrating eggs and

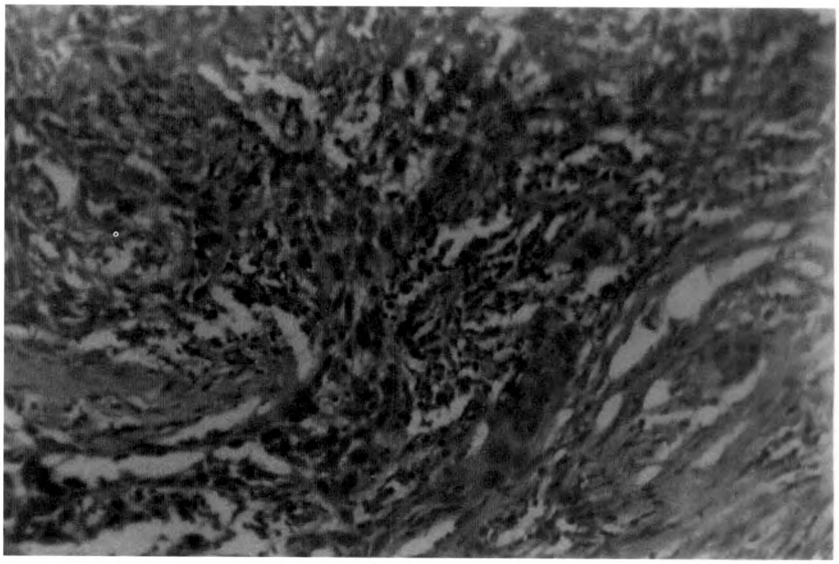

Fig 2. (Case 2) Invasive squamous cell carcinoma of larynx accompanied by stromal fibrosis (H & E, original ×20).

the constant friction of calcified ova against the basal cells of the mucous membrane.[28] Perhaps in laryngeal scleroma, there exists a similar factor in the long period of mechanical irritation of the epithelial cells

transformation of this chronic granuloma precipitated by mechanical irritation from repeated attempts at removal or by previous irradiation. But this explanation could not be suitable for the present cases,

[redacted]

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CINERADIOGRAPHY OF THE PHARYNGOESOPHAGEAL SEGMENT IN POSTLARYNGECTOMY PATIENTS

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The use of tracheoesophageal voice prostheses has gained wide acceptance in the field of vocal rehabilitation after total laryngectomy. In a randomized study with 3 arms, alaryngeal speech proficiency was assessed in 60 postlaryngectomy patients: 20 patients underwent primary unilateral pharyngeal myotomy, 21 patients underwent neurectomy of the pharyngeal plexus in addition to pharyngeal myotomy, and 19 patients did not undergo an additional surgical procedure. Pharyngoesophageal (PE) dynamics were examined during esophageal and tracheoesophageal speech. A single vibrating PE segment was seen in good alaryngeal speakers. Hypertonicity, spasm, strictures, and hypotonicity of the PE segment were correlated significantly with poor or moderate alaryngeal speech. Unilateral myotomy with or without unilateral neurectomy prevented hypertonicity or spasm of the PE segment. The acquisition of alaryngeal speech did not differ significantly between the 2 groups who had undergone an additional surgical procedure. Evaluation of anatomic and physiological factors may be helpful in subsequent clinical management to achieve effective alaryngeal speech.

KEY WORDS — alaryngeal speech, cineradiography, pharyngoesophageal segment, total laryngectomy, voice prosthesis.

INTRODUCTION

Although the survival rate after treatment of laryngeal cancer is relatively high, total surgical removal of the larynx will substantially alter speech, swallowing, and respiration. Rehabilitation of vocal functions after laryngectomy is still considered to be a major challenge. Traditionally, esophageal speech and speech by means of an artificial larynx have been the primary methods of alaryngeal vocal rehabilitation. In esophageal speech, the injection technique is routinely used to bring air into the proximal esophagus.[1] Sound is produced after this esophageal air passes through the pharyngoesophageal (PE) segment. However, the limited volume of the esophageal reservoir determines the short, quiet, low-pitched speech produced by this method.[2] In addition, only 50% to 60% of laryngectomees are able to develop esophageal speech after appropriate speech training.[3,4] The use of 1-way valved prostheses in surgically created tracheoesophageal fistulae, which enable exhaled air to be diverted from the trachea to the esophagus, has provided a more promising method of speech restoration.[5] In tracheoesophageal speech, a larger air reservoir is linked to the sound source in the PE segment.[6] Successful acquisition of alaryngeal speech has been attained via both primary and secondary puncture techniques using different types of voice prostheses.[7,8] Louder and more sustained speech may be produced. However, good tracheoesophageal speech is not invariably attained by all patients.[9] Several internal and external factors (ie, anatomic, physiological, psychological, social, and educational factors) may determine successful alaryngeal speech acquisition.[3] Altered surgical techniques and modified prosthetic devices have been introduced to facilitate tracheoesophageal speech. With the introduction of low-resistance voice prostheses, decreased intratracheal pressures during phonation were demonstrated, which resulted in more effortless expiration.[10,11] A unilateral myotomy and/or neurectomy, performed to prevent spasm and hypertonicity of the PE segment, seemed to improve esophageal and tracheoesophageal speech.[9,12-14] The function and structure of the PE segment have been assessed with air insufflation tests and radiographic contrast studies.[15-18] These dynamic studies have revealed considerable functional abnormalities, such as spasm or hypertonicity of the cricopharyngeal muscle, and structural abnormalities, such as strictures or even tumor recurrence. These disorders could interfere with successful vocal rehabilitation after laryngectomy.

The purpose of this study was to evaluate cineradiographic findings in 3 surgical groups of laryngec-

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tomees in relation to alaryngeal speech acquisition. These findings may, in addition, direct further diagnostic procedures or therapeutic interventions.

PATIENTS AND METHODS

Patients. Sixty patients (56 men and 4 women) were prospectively evaluated after total laryngectomy to assess their acquisition of esophageal speech and tracheoesophageal speech. Their ages ranged from 41 to 75 years (mean 61.2 years). The follow-up period ranged from 8 to 17 months (mean 11.4 months). All patients were treated for squamous cell carcinoma of the larynx and were irradiated before or after operation (60 to 70 Gy over 6 to 7 weeks). A unilateral radical neck dissection was performed in 24 cases.

After the total laryngectomy and primary tracheoesophageal puncture, the participating patients were randomized into 3 groups for evaluation of their alaryngeal speech proficiency. Nineteen patients (group 1) did not undergo an additional surgical procedure. A unilateral myotomy of the cricopharyngeal and inferior pharyngeal constrictor muscles was performed in 20 patients (group 2). In 21 patients, an additional unilateral neurectomy of the pharyngeal plexus was combined with a myotomy procedure (group 3).

The additional surgical procedures were performed by means of standardized techniques. After the retropharyngeal plane was entered, the unilateral myotomy was carried out on the midline of the posterior surface of the pharyngeal constrictor muscles. The myotomy was limited to the cricopharyngeal and inferior pharyngeal constrictor muscles. The vertical muscle incision was completed down to the submucosal vessels. The pharyngeal neurectomy was performed after identifying the pharyngeal plexus by electrical stimulation. The nerve plexus was approached at the level of the superior thyroid artery and the middle pharyngeal constrictor muscle. The branches were cut and separated by applying electrocautery to the edges. In all patients, a 3-layer closure of the pharyngeal defect was used, with 2 layers of inverting stitches in the mucosa and submucosa (T-shaped) and a layer of sutures in the constrictor muscle complex.

A Provox voice prosthesis (Atos Medical, Hörby, Sweden) was inserted in all 60 patients.[10] The laryngectomees were simultaneously trained to acquire both esophageal speech and tracheoesophageal speech. At the time of this study, all patients were equipped with a functional voice prosthesis.

All participating patients attended our outpatient department monthly during the follow-up period. A standardized evaluation procedure was used to compare the speech results of the laryngectomees using esophageal speech and/or tracheoesophageal speech.[19,20] This procedure was discussed at the Third International Congress on Voice Prostheses (Groningen, 1988). Semiquantitative criteria were determined for obtaining the data of the distinguished categories (phonatory skills, additional factors, general judgment). This assessment enabled differentiation among good, moderate, and poor speakers. As the vocal performance of tracheoesophageal speech differs from esophageal speech, separate criteria were applied to assess the 2 speech modes.[20]

Cineradiographic Evaluation. Cinepharyngoesophagograms and static radiographs were used to evaluate the radiologic characteristics of the PE segment in all 60 patients. Cinematography allowed examination of the movement of the reconstructed pharynx, while the spot films showed a more morphologically detailed but static image of the pharynx and PE segment. All examinations were performed by a team of permanent medical employees consisting of a radiologist, a speech therapist, and an otorhinolaryngologist. A standard Siemens remote-controlled fluoroscopy unit with the table in a vertical position was used while the patients were sitting. The distance between the table and the diaphragm of the X-ray tube measured 45 to 50 cm, which allowed examination of the patient in a sufficiently lateral position with the shoulders slightly oblique. Lateral pulsed cineradiography was combined with anteroposterior films (100 frames per second) to further evaluate presenting morphological and functional abnormalities. Barium sulfate (E-Z Paque HD) was used to sufficiently coat and delineate the oral, pharyngeal, and esophageal mucosa. The tracheostoma was identified by a radiopaque marker at the upper tracheocutaneous border. The Provox voice prosthesis could be easily localized by the radiopaque ring at the esophageal end.

Before fluoroscopy, each patient was instructed to keep a fixed amount of barium (10 mL) in the mouth while the pharynx was viewed in lateral and anteroposterior positions. During the swallowing act, attention was focused on the movements of the tongue base, anatomic and functional abnormalities of the neoglottis, presence of a pseudodiverticulum, and relaxation of the upper esophageal sphincter. Before attempting esophageal phonation (/a/ and /e/ at comfortable and maximal intensity levels), the patients used the injection technique to inject small amounts of air into the esophagus. Tracheoesophageal speech was attempted after manual closure of the tracheostoma and sufficient inspiration. During phonation (40 to 60 dB hearing level), the characteristics (regular,

TABLE 1. CINERADIOGRAPHIC FEATURES USED TO EVALUATE FUNCTIONAL AND STRUCTURAL CHANGES OF PE SEGMENT DURING ALARYNGEAL SPEECH AT NORMAL INTENSITY LEVELS (40 TO 60 DECIBELS)

	Esophageal Phonation	*Tracheoesophageal Phonation*
Normal	Normal passage of barium during swallowing, proximal esophageal air reservoir with regular vibrating PE segment, good voice	Similar features
Hypertonicity	Normal passage of barium, narrowed or divided PE segment, irregular vibrations, regular air reservoir, poor to moderate voice	Similar features, improved voice quality during relaxed and slow attempts, dilated air reservoir and esophagus (propulsion)
Spasm	Slightly delayed passage of barium after multiple swallows, severely narrowed or divided PE segment with bulging pseudoglottis posteriorly, small variable air reservoir, poor voice	Narrowed and elongated PE segment, widely dilated air reservoir with marked propulsion, no voice (only sudden explosive releases)
Hypotonicity	Dilated pharynx during swallowing and attempted phonation, no typical PE segment with minimal vibrations, poor voice	Similar features, large dilated pharynx, whispery voice, improved speech after digital pressure applied to neck
Stricture	Holdup of barium above persistent narrowed segment, absent air reservoir, no voice	Similar features, dilated esophagus, no relaxation of pharynx, poor or no voice

PE — pharyngoesophageal.

irregular, absent) of the vibrations of the PE segment, as well as the location of the upper and lower segments of the vibrating PE segment, were determined. The size of the air reservoir in the proximal esophagus was estimated (related to the number of corresponding cervical vertebrae), and attention was given to the amount of air that escaped to the distal esophagus (propulsion). The neopharynx was screened for possible spasm of the upper esophageal sphincter, strictures, and hypertonicity or hypotonicity of the PE segment (Table 1).[16]

Data Analysis. The speech and PE segment data were analyzed statistically by using repeated measures analysis of variance (ANOVA) to identify significant differences among the 3 groups and the alaryngeal speech method. All possible interactions among the surgical groups, speech outcome, and PE segment characteristics were tested separately. We performed χ^2 comparisons to demonstrate the source of significance for all variables with main-effect ANOVA results that had p values of less than .05. To verify the reliability of measures used in this study, we randomly reanalyzed speech and cinematography data for 20 patients. Interjudge and intrajudge differences were distinguished by assessment of the respective κ values of the specific variables. Analyses were performed with the Scientific Package for Social Sciences (SPSS).

RESULTS

Speech Results. The 3 groups of laryngectomees were comparable with respect to age, sex, and additional neck dissection. No significant interactions were observed for esophageal speech or tracheoesophageal speech based on age and sex differences ($p > .005$). The results of esophageal and tracheoesophageal speech are given in Table 2. Overall esophageal speech was rated good in 20% (n = 12) of all cases. Moderate esophageal speech was observed in 57% (n = 34), whereas unsuccessful esophageal speech was found in 23% (n = 14) of the laryngectomees. Esophageal speech results differed significantly among the 3 groups ($p = .002$). The majority of patients in group 1 (85%) did not acquire fluent esophageal speech. Functional esophageal speech results were obtained in 85% and 95% of the patients in the surgical groups with additional myotomy and combined myotomy and neurectomy procedures, respectively, compared to 47% in the group of laryngectomees in which these procedures were not performed. The results of tracheoesophageal speech were better in all surgical groups, compared to esophageal speech (Table 2). Tracheoesophageal speech was rated good in 58%, whereas 30% of the laryngectomees obtained moderate results. Laryngectomees who underwent primary myotomy and neurectomy procedures (group 3) were most successful in obtaining good tracheoesophageal speech (81%). However, functional tracheoesophageal speech was obtained in the majority of patients (95%) in groups 2 and 3. Most tracheoesophageal speech failures were observed in group 1 (26%). These differences were significant ($p < .0001$) across the surgical groups.

Cineradiographic Characteristics. Radiographic contrast studies in the early postoperative period demonstrated pharyngocutaneous fistulae in 9 patients (15%; Table 3). The majority of these fistulae (n = 5, 26%) occurred in laryngectomees from group 1. No significant differences ($p = .526$) were apparent between the surgical groups. Six patients developed a pharyngeal stricture at the level of the former fistula tract. A pseudoepiglottis was noticed in 93% of all patients during attempted phonation, at the anterior wall of the PE segment, just inferior to the base of

TABLE 2. RESULTS OF ESOPHAGEAL SPEECH AND TRACHEOESOPHAGEAL SPEECH AMONG THREE SURGICAL GROUPS

		Speech					
		Poor		*Moderate*		*Good*	
Group	*n*	*n*	*%*	*n*	*%*	*n*	*%*
Esophageal speech							
1	19	10	52.6	6	31.5	3	15.8
2	20	3	15.0	15	75.0	2	10.0
3	21	1	4.8	13	61.9	7	33.3
All	60	14	23.3	34	56.7	12	20.0
Tracheoesophageal speech							
1	19	5	26.3	8	42.1	6	31.6
2	20	1	5.0	7	35.0	12	60.0
3	21	1	4.8	3	14.3	17	81.0
All	60	7	11.7	18	30.0	35	58.3

Group 1 — laryngectomy with no additional surgical procedure; group 2 — unilateral myotomy of cricopharyngeal and inferior pharyngeal constrictor muscles; group 3 — myotomy with additional unilateral neurectomy of pharyngeal plexus.

TABLE 3. STRUCTURAL ASPECTS OF PE SEGMENT DETERMINED DURING CINERADIOGRAPHY

	Group						
	All *(n = 60)*	*1* *(n = 19)*		*2* *(n = 20)*		*3* *(n = 21)*	
	n	*n*	*%*	*n*	*%*	*n*	*%*
Previous fistulae*	9	5	26.3	3	15.0	1	4.8
Pseudo-epiglottis†	56	17	89.5	19	95.0	20	95.2
Broad pseudoglottis size§	19	13	68.4	5	25.0	1	4.8
Bilobular pseudoglottis shape§	46	9	47.4	17	85.0	20	95.2

See Table 2 for definition of groups.

*Remnant of previous pharyngocutaneous fistula.

†Anterior pouching of pharyngeal wall.

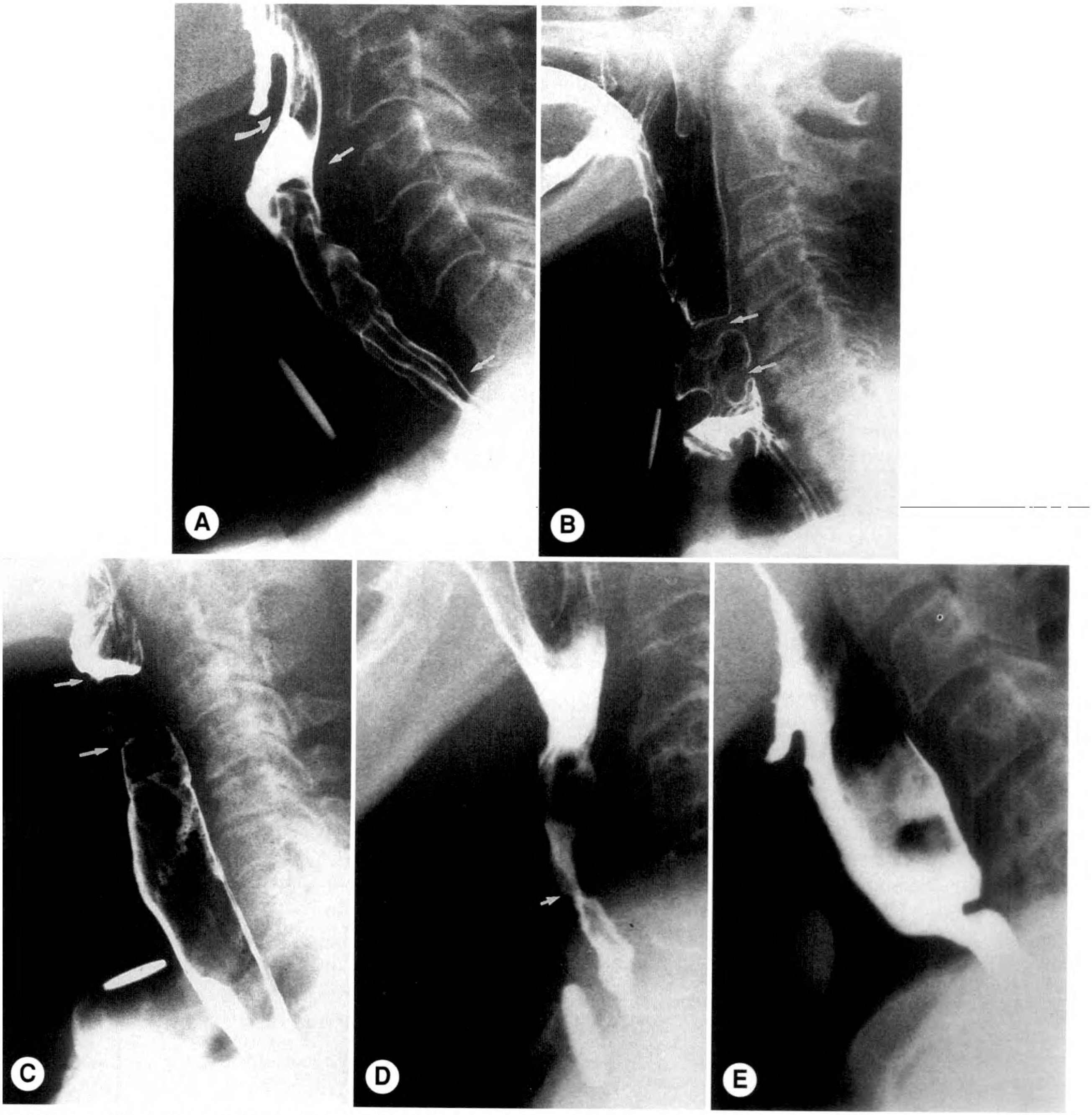
§Size and shape of upper esophageal sphincter.

the tongue (see Figure, A). No significant difference (p = .749) in occurrence was observed between good and poor alaryngeal speakers. This structure may not be related to voice quality. A broad pseudoglottis (width of more than 1 cervical vertebra) with evidence of spasm or hypertonicity of the upper esophageal sphincter was found in 19 cases. This feature appeared to be significant (p = .0002) for voice failures in group 1. A significant correlation (p < .001) between poor esophageal speech and a delayed opening of the upper esophageal sphincter was demonstrated. A bilobular aspect to the PE segment was associated with functional esophageal speech in all surgical groups. The Provox voice prosthesis was located inferior to the pseudoglottis in all cases. Tracheoesophageal speech failure was not associated with obstruction of the esophageal part of the prosthesis.

On average, the inferior level of the vibrating PE segment was situated during phonation at C6 (Table 4). No significant (p > .005) differences concerning the quality of esophageal or tracheoesophageal speech were observed for this inferior level. However, the superior part of the PE segment was more variable. During esophageal phonation, no significant differences (p > .005) were found for this feature among the 3 surgical groups, although a tendency toward a more superiorly located upper level (mean level of cervical vertebrae 4.3 ± 0.7) was evident in good esophageal speakers. Fluent tracheoesophageal speakers showed a significantly (p = .0001) higher upper level of the vibrating PE segment (mean level of cervical vertebrae 3.3 ± 0.5). A vibrating PE segment that was more elongated during attempted phonation seemed to be correlated with good esophageal and tracheoesophageal speech.

Spasm, hypertonicity, and hypopharyngeal strictures were significantly (p < .005) associated with both esophageal and tracheoesophageal speech failures (Table 5). During attempted tracheoesophageal speech, most structural and functional disorders were more prominently demonstrated (see Figure, B-E). Most of these disorders differed significantly (p = .0001) among the 3 groups, analogous to the speech results. Most disorders were assessed in group 1. Only 1 patient (group 3) with moderate tracheoesophageal speech showed a hypotonic segment. Vibratory movements of the PE segment during tracheoesophageal speech differed significantly (p = .0001) among the surgical groups (Table 6). A significant interaction between vibratory movements and speech results was observed in group 1 (p = .0001) and group 2 (p = .0002). Similar results were found with regard to the size of the air reservoir during phonation. Good esophageal and tracheoesophageal speakers showed a prominent air reservoir during phonation. A small or absent air reservoir was more obvious in laryngectomees with poor or moderate esophageal speech. Abundant propulsion of air toward the distal esophagus was only evident in poor tracheoesophageal speakers, suffering from strictures, hypertonicity, or spasm of the PE segment, in groups 1 and 2. During attempted esophageal phonation, no significant (p = .13) interactions were observed between the occurrence of propulsion of air and speech outcome.

The voice and cineradiography data of 20 patients were randomly reanalyzed to verify the reliability of the measures. Intrajudge reliability between the measures ranged from 0.46 to 1.0 (mean, 0.66). The inter-



Radiographic contrast studies. A) Lateral view of laryngectomee with good tracheoesophageal speech during phonation. Upper and lower levels of vibrating pharyngoesophageal (PE) segment are marked with straight arrows. Pseudoepiglottis is present (curved arrow). B) Hypertonicity of PE segment during attempted tracheoesophageal phonation. Narrowed segments are outlined by arrows. C) Spasm of PE segment during attempted tracheoesophageal phonation. Narrowed segment is marked with arrows. Below spastic segment, dilated proximal esophagus is visible. D) Narrowing of PE segment by stricture (arrow) that failed to dilate during swallowing or phonation. E) Hypotonicity of PE segment. Dilated pharynx is visible without vibratory movements of pharyngeal wall.

udge reliability between the measures ranged from .51 to 1.0 (mean, 0.68). Intrajudge and interjudge neasures were satisfactory.

DISCUSSION

Tracheoesophageal speech, in combination with sophageal speech, has become an established meth- d of alaryngeal voice rehabilitation. Most patients acquire fluent tracheoesophageal speech for effective daily communication without specific intervention.[5,7,8] However, a significant number of patients still fail to achieve effective esophageal or tracheoesophageal speech. Several authors have reported failure rates varying from 10% to 80%.[3,9,21] In this study, laryngectomees who underwent a primary myotomy procedure or a combined myotomy and neurectomy

TABLE 4. EVALUATION OF UPPER AND LOWER LEVELS OF VIBRATING PE SEGMENT DURING ESOPHAGEAL SPEECH AND TRACHEOESOPHAGEAL SPEECH

	Speech Quality					
	Poor		*Moderate*		*Good*	
Group	*Lower*	*Upper*	*Lower*	*Upper*	*Lower*	*Upper*
Esophageal speech						
1	5.9 ± 0.3	4.8 ± 0.4	5.7 ± 0.5	4.7 ± 0.5	6.0 ± 0.1	4.2 ± 0.4
2	6.0 ± 0.2	4.7 ± 0.5	5.9 ± 0.2	4.6 ± 0.4	6.0 ± 0.1	4.3 ± 0.4
3	6.0 ± 0.2	5.0 ± 0.1	6.0 ± 0.3	4.7 ± 0.4	6.2 ± 0.2	4.2 ± 0.5
Tracheoesophageal speech						
1	6.1 ± 0.5	5.0 ± 0.4	6.3 ± 0.7	4.2 ± 0.4	5.8 ± 0.3	4.1 ± 0.6
2	6.0 ± 0.1	5.0 ± 0.3	5.9 ± 0.4	4.4 ± 0.5	6.1 ± 0.4	3.4 ± 0.5
3	6.0 ± 0.3	4.1 ± 0.2	6.0 ± 0.3	3.5 ± 0.4	6.4 ± 0.8	3.3 ± 0.4

Data are cervical vertebra level ± SD.

procedure at the time of laryngectomy demonstrated better esophageal and tracheoesophageal speech proficiencies (60% to 80% and 10% to 33%, respectively) compared to laryngectomees who had not undergone these additional procedures (32% and 16%, respectively).

Removal of the larynx is a major disabling procedure, and failure of vocal rehabilitation is often related to psychosocial problems. These problems appear to be responsive to active intervention.[4] In the case of tracheoesophageal speech failure, device-related causes should be excluded before further investigations are conducted.[22] In all patients, the Provox voice prosthesis appeared to be localized below the PE segment. No obstruction of airflow due to device-related problems or blockage of the valve part by the posterior wall of the esophagus was apparent.

Physical factors that influence vibratory characteristics of the PE segment seem to affect esophageal speech acquisition directly. In assessing the quality of speech, the reconstructed PE segment should be examined dynamically.[23] Fluoroscopy can be used to detect functional or morphological abnormalities within the oral, pharyngeal, and esophageal segments. Patients with functional tracheoesophageal speech showed a regular-vibrating PE segment during phonation. The vibrating segment appeared to be more elongated in good shunt esophageal speakers. In the majority of examined patients, the "pseudoglottis" was located at the junction of the neopharynx and upper esophagus at the level of the fifth and sixth cervical vertebrae. In fluoroscopic and strobofibroscopic studies, the "pseudoglottis" has been recognized as a protrusion of the posterior wall at the lower PE segment.[18] The inferior level of the PE segment did not differ among poor or proficient shunt and injection esophageal speakers. The upper part of the pseudoglottis is considered to be the sound-producing part, because during phonation, a regular vibration occurs in conjunction with that of the proximal part of the PE segment, while the lower part shows no regular vibration.[23]

TABLE 5. EVALUATION OF FUNCTIONAL AND STRUCTURAL ABNORMALITIES OF PE SEGMENT DURING ESOPHAGEAL SPEECH AND TRACHEOESOPHAGEAL SPEECH

	Groups						
	1-3	*1*		*2*		*3*	
	p	*n*	*p*	*n*	*p*	*n*	*p*
Esophageal speech							
Spasm	.001	4	.001	2	.082	0	
Hypertonicity	.0002	9	.0007	8	.001	0	
Hypotonicity	.5832	0		0		1	.349
Stricture	.0089	3	.002	2	.03	1	.008
Tracheoesophageal speech							
Spasm	.0001	6	.0001	4	.0006	0	
Hypertonicity	.0001	7	.0001	3	.0006	1	.818
Hypotonicity	.022	0		0		1	.818
Stricture	.0001	3	.0001	2	.0001	1	.0001

Statistical analyses (χ^2) were performed to determine any interaction with speech failure ("1-3" is interaction between surgical groups).

After total laryngectomy, the size and shape of the pharynx have changed so that it is a more straight and tubular structure. At the upper surgical margin the neopharynx may appear narrower, and the formation of a pseudodiverticulum or pseudoepiglottis at the anterior wall may be observed. This sharp-angled structure is located anteriorly at the upper suture line of the neopharynx. Widening below this junction may produce the impression of an "epiglottis." Approximately 90% of our patients showed such a pseudodiverticulum. Its existence might be related to postoperative altered pharyngeal physiology or to the method of pharyngeal closure. In other studies, these diverticulae are reported to occur in 35% to 100% of laryngectomees.[24] However, these structures do not interfere with alaryngeal speech proficiency. Hypopharyngeal strictures, PE spasm, hypertonicity,

TABLE 6. ASSESSMENT OF SPECIFIC CHARACTERISTICS OF PE SEGMENT DURING ESOPHAGEAL SPEECH AND TRACHEOESOPHAGEAL SPEECH

	Esophageal Speech			*Tracheoesophageal Speech*		
Group	*1*	*2*	*3*	*1*	*2*	*3*
Vibration	.0009	.0001	.1539	.0001	.0002	.2825
Reservoir	.0047	.0820	.6810	.0001	.0007	.8838
Propulsion	.2461	.8819	.4076	.0001	.0003	.7395

Interactions (p value) between specific characteristics and alaryngeal speech proficiency are classified by surgical group.

and hypotonicity of the PE segment have been identified as considerable causes of speech failure after total laryngectomy. More than 70% of our patients with hypopharyngeal strictures (n = 6) had suffered from early postoperative pharyngocutaneous fistulae, which delayed the postoperative rehabilitation period. In 4 patients, repeated PE dilatations resolved these problems related to hypopharyngeal stenosis. In the literature, pharyngocutaneous fistulae are reported to occur in 5.9% to 37.6% of patients after total laryngectomy.[25,26] Pharyngeal disruption with fistula formation is one of the most vexing problems resulting from laryngectomy, because it may be chronically associated with delayed healing. Besides delayed healing, the end result of fistula formation is, commonly, scarring and stricture of the pharynx. In our series, the laryngectomees who had undergone a unilateral myotomy with or without a neurectomy of the pharynx did not demonstrate a higher incidence of postoperative fistulae or strictures.

Pharyngoesophageal spasm and hypertonicity remain the most common reasons for esophageal and tracheoesophageal speech failure (38% and 35%, respectively). Spasm of the upper esophageal sphincter as a possible cause of dysphagia and alaryngeal speech failure was first described by Schobinger[27] in 1958. The upper esophageal sphincter is innervated by pharyngeal branches of the vagus nerve, which regulates resting tone, relaxation, and contraction. Normally, bilateral innervation causes constriction of the constrictor muscles and relaxation of the cricopharyngeal muscle. At rest, the reverse is true. Impairment of the sphincter function may be due to damage to local vagal branches during surgery. This may result in a decreased resting pressure of the cricopharyngeal muscle with uncoordinated contractions of the constrictor muscles.[28] Spasm in the postlaryngectomy patient appears to be a normal reaction to esophageal distention that prevents gastroesophageal reflux. The majority of speech failures related to a disturbed tonicity of the PE segment were found in the group of laryngectomees that did not undergo additional surgical procedures (group 1). Although a statistically lower incidence of spasm and hypertonicity ($p = .001$) was demonstrated in the combined myotomy-neurectomy group, compared to the myotomy group, no significant difference in speech results ($p > .05$) was observed among these groups. The benefit of a combined procedure was not clear with regard to the acquisition of successful alaryngeal speech. The value of an additional neurectomy procedure to prevent pharyngospasm during alaryngeal phonation should be assessed separately. In 5 cases, we performed a secondary pharyngeal constrictor myotomy with successful postoperative tracheoesophageal speech acquisition. A secondary neurectomy is more difficult to perform, because nerve identification might be difficult or impossible after previous dissection and scarring have occurred in the region. A percutaneous pharyngeal nerve block may precede any surgical procedure to confirm a disturbed tonicity of the PE segment in relation to voice failure. An alternative treatment option consists of using botulinum neurotoxin injections at the area of the pharyngeal constrictors to facilitate relaxation of the PE segment and improve alaryngeal phonation.[29] However, hypotonicity of the PE segment may occur as a consequence of these additional procedures.[12] Only 1 laryngectomee in our study suffered from a weak and breathy voice due to a hypotonic PE segment. Typically, the anterior and posterior walls of the reconstructed pharynx appear widely separated during attempted phonation. Only after external pressure was applied to the anterior neck could a vibrating segment be visualized. It has been strongly recommended that only a unilateral myotomy of the lower part of the neoglottis, which is considered to be the cricopharyngeal muscle,[9] be performed. By conserving the pharyngeal constrictor muscles, the vibrating segment of the neoglottis is kept functional, while total flaccidity of the PE segment is prevented. In cases of low pharyngeal wall tension, a standard "high"-resistance voice prosthesis could contribute to a more acceptable voice by limiting the diversion of expiratory air into the pharynx.

Some authors believe that successful speech will be attained with time and that poor tracheoesophageal speakers will profit from speech training and practice without specific surgical interventions to lower the resting tone of the upper esophageal sphincter.[30] After an appropriate period of time (>6 months), the esophagus may become accommodated to the insufflation of air, resulting in decreased spasm. Adaptation of the esophageal reflexes or extended speech practice may improve speech results, despite high esophageal pressures. Patients will also gradually improve their ability to occlude the tracheostoma in an appropriate way without excessive pressure, which will optimize airflow through the prosthesis.[3] In our series, the laryngectomees with poor speech results due to factors related to the tonicity of the PE segment did not succeed after a relatively long period of time (mean 14.2 months), despite proper speech training.

Dynamic radiographic imaging of the PE segment following laryngectomy may reveal a broad spectrum of functional and morphological aspects. During functional alaryngeal phonation, a bilobular PE segment with a regular vibratory pattern is visualized during both esophageal and tracheoesophageal speech. Unsuccessful alaryngeal speech may be caused by several internal and external factors. Evaluation of poor alaryngeal speech is performed by successively checking patient- and device-related problems. Pharyngoesophageal spasm was the most common reason for esophageal speech failure, followed by hypertonicity and hypopharyngeal strictures. These disorders were most clearly demonstrated during tracheoesophageal speech attempts. The pharyngeal constrictor myotomy and pharyngeal plexus neurectomy, which are performed to prevent muscle spasm and hypertonicity of the neoglottis, may promote successful acquisition of early esophageal and tracheoesophageal speech. However, the beneficial role of an additional neurectomy procedure in patients undergoing total laryngectomy with a unilateral myotomy of the PE segment was not clear. In our opinion, a total laryngectomy procedure should primarily include a unilateral myotomy of the PE segment to facilitate alaryngeal speech. Cineradiography is a suitable method for demonstrating functional and structural disorders that may be responsible for swallowing difficulties or alaryngeal speech failures. These imaging studies allow proper management of patients who undergo laryngectomy.

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DYSPHAGIA AFTER RADIOTHERAPY: ENDOSCOPIC EXAMINATION OF SWALLOWING IN PATIENTS WITH NASOPHARYNGEAL CARCINOMA

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Dysphagia is a late sequela compromising the lives of more than one fourth of patients with nasopharyngeal carcinoma (NPC) who survive long after radiotherapy. By using fiberoptic endoscopic examination of swallowing as a modality for dysphagia evaluation, we were able to easily recognize the functional and anatomic changes in 31 dysphagic NPC patients. The majority of patients were found to aspirate after the act of swallowing (77.4%). Seventeen (54.8%) had tongue atrophy, and 9 (29%) had vocal cord palsy. Dysfunctions such as dry mouth (45.2%), velopharyngeal incompetence (58%), premature leakage (41.9%), delay or absence of swallow reflex (87.1%), poor pharyngeal constriction (80.6%), pharyngeal residue retention (83.9%), penetration or aspiration (93.5%), and silent aspiration (41.9%) were noted in these patients. Multiple dysfunctions were demonstrated in each patient. Abnormality of pharyngeal constriction and/or aberrant upper esophageal sphincter function played the major role in the postswallow aspiration of these irradiated NPC patients. Clinically compromised patients (weight loss of ≥5 kg in 1 year or pneumonia) had more of the above anatomic and functional impairments. The radiation dosage and volume bore no correlation to the time of symptom onset, or to the occurrence of functional changes.

KEY WORDS — dysphagia, fiberoptic endoscope, nasopharyngeal carcinoma, radiotherapy.

INTRODUCTION

With the use of radiotherapy, a marked cure rate is obtained and long-term survival is achievable for patients with nasopharyngeal carcinoma (NPC).[1] In treating this disease with irradiation, normal adjacent tissue of the head and neck is inevitably exposed to radiation. Various late sequelae such as chronic otorrhea, trismus, and osteoradionecrosis ensue and last as long as the patient is alive. These complications compromise the survivor's quality of life and are sometimes life-threatening. Dysphagia or choking is one of these sequelae, to which little attention had been paid, although 27.2% of irradiated patients in one study who survived NPC were bothered by this disorder.[2]

Dysphagia in irradiated NPC patients also raises the suspicion of tumor recurrence. After the possibility of recurrence is ruled out, anatomic and functional lesions caused by radiation should be studied to account for this formidable disorder. To our knowledge, few investigations have been done on deglutition dysfunction of irradiated NPC patients. Because fiberoptic endoscopic examination of swallowing (FEES) has been shown to be a handy alternative to videofluoroscopy for monitoring deglutition function,[3-5] we carried out this study to investigate the anatomic and functional changes in dysphagic NPC patients with emphasis on FEES as a modality of assessment. Another major purpose of this study was to identify uniform or specific abnormalities in these irradiated patients that warrant special surgical treatment or rehabilitation programs.

MATERIALS AND METHODS

Thirty-one patients with NPC who were treated successfully with radiotherapy and complained of deglutition disorders were referred to the dysphagia team. After a comprehensive study to rule out the possibility of recurrence or metastasis, a total of 31 patients (all Taiwanese) were collected. The sex distribution was 16 men and 15 women, and their ages ranged from 33 to 78 years (mean 54.2 years). The initial clinical stage based on our criteria[6] ranged from I to III, with the exclusion of patients with cranial nerve involvement. None of them had complaints of swallowing disturbances or presentations of tongue atrophy and vocal cord palsy before treatment. The irradiation dosage ranged from 6,900 to 8,000 cGy to the nasopharynx, and from 5,000 to 7,100 cGy to the neck, according to the extent and response of the lesions. The treated volume covered a column from the basal skull down to the clavicle level. The initial portal sizes were individualized; however, there was little difference. After an initial dosage of 3,600 cGy

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in 18 fractions, 4 small portals (2 laterals of 7 × 9 cm and 2 anterior obliques of 5 × 6 cm) were focused on the nasopharynx, and large anterior and posterior portals with central shields were focused onto bilateral cervical lymphatic chains. During the course of treatment, the irradiation field always covered the whole neck and the lower part of the oropharynx except for a small strip of the central portion, no matter what the size of neck metastasis was.[1] Dysphagia resulting from mucositis during and immediately after radiotherapy was excluded. The time intervals between radiotherapy and the development of significant deglutition problems ranged from 2 to 20 years (mean 8.5 years). After a comprehensive study to rule out the possibility of recurrence or metastasis, all the patients underwent FEES with test substances of 2 different consistencies (pudding and liquid dye).

For FEES,[7] the subjects were in the postures in which they normally ate. Tongue motion and soft palate elevation were first inspected. After appropriate topical anesthesia of the nasal cavity, a fiberoptic rhinolaryngoscope (Machida ENT30RIII) attached to a CCD camera (Karl Storz 9050NC) and a color video monitor was inserted gently through the nasal floor. With the scope viewing the nasopharynx, the subject was asked to dry swallow to allow assessment of velopharyngeal competence. The scope was then deflected downward and passed on into the oropharynx to a position from which the whole laryngopharynx could be viewed panoramically. The general appearance of the pharynx and larynx was noted. The adequacy of vocal cord movement was assessed during phonation and inspiration. Pooling or aspiration of saliva was also documented, if any. A dry swallow was requested again, and pharyngeal constrictor competence was assessed. At the same time, laryngeal elevation could be evaluated by inspection and palpation.

After completing the preswallowing assessment, we proceeded to a sequence of swallows of pudding and oral liquid dye. A spoonful of pudding (3 mL) was first administered, and the subject was instructed to hold the bolus in the mouth for 10 seconds. Any premature oral leakage to the pharynx or even preswallowing aspiration was well observed under the endoscope. After moderate chewing, the subject was asked to swallow in a normal manner. Assessment was made on observations just before the initiation of swallowing and immediately after the swallowing. The lateralization of food passage or stasis was also documented. Focus was made on laryngeal penetration or tracheal aspiration in this process. If significant pharyngeal residue was noted, the observation was continued for 1 to 2 minutes after swallowing, to determine whether spillover ensued. In total, 3 spoonfuls of pudding were given in sequence, and the procedure was terminated once aspiration occurred. The response to aspiration and the efficiency of the cough reflex were also noted. Another 3 boluses of 3 mL of diluted green food color dye were then administered to subjects who safely passed the above examination. Procedures were terminated if any aspiration occurred.

All procedures were performed by laryngologists and speech therapists of the dysphagia team. All the observations made under the endoscope were documented with videotapes.

RESULTS

All patients except 2 complained of swallowing difficulty for liquids. Dysphagia for dry solid food was reported in 12 patients. Five had been hospitalized due to aspiration pneumonia. Weight loss was common, with an average of 3.4 kg in 1 year, though it was not necessarily caused by swallowing problems.

Figure 1 illustrates the time lag of dysphagic symptoms in relation to the dosage of radiation to the nasopharynx and neck. There was no apparent correlation between the dosage of radiation exposure and the time from radiotherapy to the onset of deglutition problems (Pearson correlation test).

Assessment was made clinically according to the identification of aspiration before, during, or after the swallowing. Under the endoscope, only 3 patients (9.7%) showed preswallowing aspiration. Nineteen (61.3%) aspirated during the act of swallowing. The majority of aspiration events (77.4%) occurred during the observation period after the initial swallow action.

Anatomic changes related to dysphagia were documented, with emphasis on tongue motion and vocal cord function. Neck stiffness was not evaluated due to lack of an objective reference. Seventeen patients (54.8%) were noted to have tongue atrophy at least unilaterally. Bilateral tongue lesions were noted in 7 of them. Six showed fasciculation of the atrophic tongue. Vocal cord palsy or paresis was found in 9 patients (29%). None of these physical impairments were present before irradiation.

Eight functional aspects were documented: dry mouth, velopharyngeal incompetence, premature leakage, delay or absence of swallow reflex, poor pharyngeal constriction, pharyngeal residue retention, laryngeal penetration or aspiration, and cough response. Fourteen patients (45.2%) had dry mouth, 18 (58.1%) had nasopharyngeal regurgitation, 13 (41.9%) had premature leakage, 27 (87.1%) suffered from delay

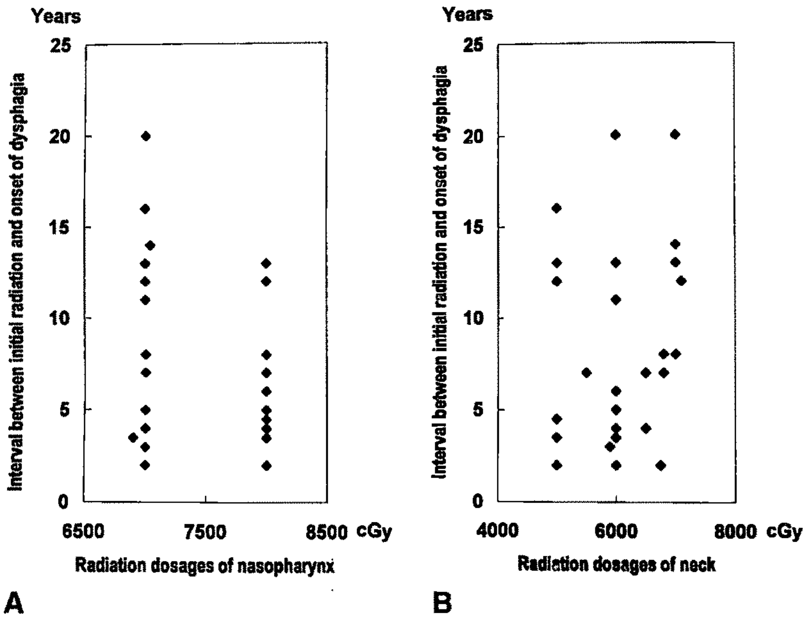

Fig 1. Time-dose relationships between latency interval before onset of dysphagia and A) radiation exposure to primary focus (nasopharynx) and B) radiation exposure to neck.

or absence of the swallow reflex, 25 (80.6%) had weakness of the pharyngeal constrictors during swallowing, and 26 (83.9%) had laryngeal penetration or tracheal aspiration, with 13 of them revealing silent aspiration. The most prominent pharyngeal dysfunction among these patients was partial bolus retention. All patients but 2 (93.5%) showed stasis of bolus residue at the inlet of the esophagus immediately after the first act of swallow.

Eleven patients were found to be clinically compromised (defined as weight loss of ≥5 kg in 1 year and/or aspiration pneumonia). A higher tendency toward occurrence of the above anatomic and functional impairments was found in this group, except for xerostomia and velopharyngeal incompetence. All suffered from postswallow aspiration and revealed pharyngeal retention on examination. Scoring of the number of items of impairments showed significantly high scores for the compromised group (Table 1). No difference in the radiation dosages was found between the compromised and compensated groups.

Multiple pharyngeal dysfunctions were demonstrated in each dysphagic patient (Table 2). Among them, good correlation was found between vocal cord palsy and aspiration during swallowing ($p < .05$, χ^2 test). Both poor pharyngeal constriction and pharyngeal retention were also found to correlate with the occurrence of postswallow aspiration ($p < .05$, χ^2

TABLE 1. ANATOMIC AND FUNCTIONAL IMPAIRMENTS AND RADIATION DOSAGES IN GROUPS WITH DIFFERENT CLINICAL OUTCOMES

Swallowing Function		*Compromised (n = 11)*	*Compensated (n = 20)*
Aspiration	Before	3 (27.3%)*	0*
	During	9 (81.8%)	10 (50%)
	After	11 (100%)*	13 (65%)*
Deglutition dysfunction	Dry mouth	4 (36.4%)	10 (50%)
	Premature leakage	6 (54.5%)	7 (35%)
	Velopharyngeal incompetence	6 (54.5%)	12 (60%)
	Delayed or absent reflex	10 (90.9%)	17 (85%)
	Poor pharyngeal constriction	10 (90.9%)	15 (75%)
	Pharyngeal retention	11 (100%)	18 (90%)
	Aspiration	11 (100%)	15 (75%)
	Silent aspiration	6 (54.5%)	7 (35%)
Anatomic change	Tongue atrophy	9 (81.8%)	8 (40%)
	Vocal cord palsy	5 (45.5%)	4 (20%)
Impairment score (ie, No. of impaired items)		7.09†	5.65†
Radiation dosage (cGy)	Nasopharynx	7,358	7,450
	Neck	6,000	6,042

*$p < .05$ (χ^2 test).
†$p < .05$ (Student's *t*-test).

TABLE 2. CASE DISTRIBUTION OF VARIOUS PHARYNGEAL DYSFUNCTIONS (N = 31)

Pharyngeal Dysfunction	*Velopharyngeal Incompetence*	*Delayed or Absent Swallow Reflex*	*Poor Pharyngeal Constriction*	*Pharyngeal Residue Retention*	*Penetration or Aspiration*	*Vocal Cord Palsy*
Velopharyngeal incompetence (n = 18)						
Delayed or absent swallow reflex (n = 27)	14*					
Poor pharyngeal constriction (n = 25)	13	24*				
Pharyngeal residue retention (n = 29)	16	25	24			
Penetration or aspiration (n = 26)	15	23	23†	24†		
Vocal cord palsy (n = 9)	6	9	9	8	9§	

*$p < .05$, χ^2 test.

†Correlated with aspiration after swallowing, $p < .05$, χ^2 test.

§Correlated with aspiration during swallowing, $p < .05$, χ^2 test.

test). Velopharyngeal incompetence and poor pharyngeal constriction were found to be correlated with delayed or absent swallow reflex ($p < .05$, χ^2 test). However, none of these dysfunctions, nor the magnitude of weight loss, correlated with the initial radiation dosage (Student's *t*-test and Pearson correlation). There was no relationship between these dysfunctions and initial clinical staging (χ^2 test).

DISCUSSION

As irradiated NPC patients survive longer, the number of patients suffering from severe dysphagia will increase. However, there is little information regarding the pathophysiology of this specific disorder. Our findings revealed that the most common dysfunction of deglutition in these patients was pharyngeal retention (93.5%), which correlated well with the occurrence of postswallow aspiration (Table 2). In the absence of compensatory protective maneuvers, bolus retained in the hypopharynx might easily spill into the laryngeal aperture following the swallow (Fig 2). Possible causes related to the increased

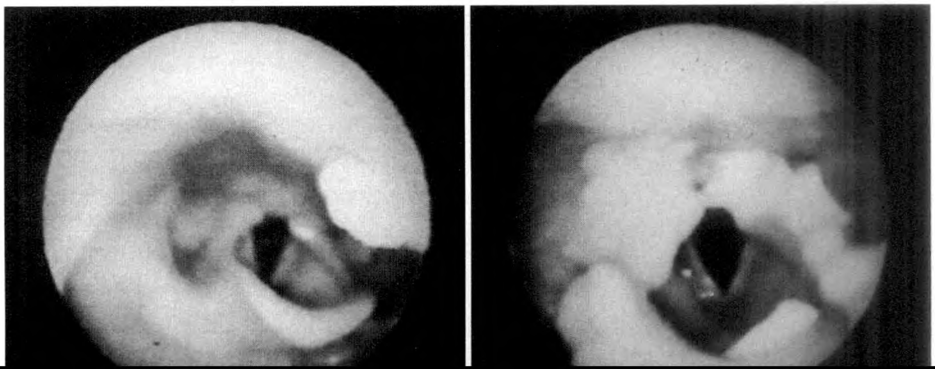

Fig 2. Swallowing test in an irradiated nasopharyngeal carcinoma

[redacted]

TABLE 3. MERITS OF FIBEROPTIC ENDOSCOPIC EXAMINATION OF SWALLOWING AS COMPARED TO VIDEOFLUOROSCOPY

- Advantages of FEES
  - Availability
    - Less staff and space needed
    - Transportable
    - Testing materials commercially available
  - Safety
    - Easy rescue of pharyngeal residue
    - Nonirradiative
  - Sensitivity
    - 3-D panoramic view
    - Repeatable
    - Less sampling error
  - Efficiency
    - Short analysis time
    - Biofeedback possible
- Disadvantages of FEES
  - Loss of vision at height of pharyngeal swallowing
  - Quantitative analysis unavailable
  - Limited information on oral and esophageal phases
- Common deficits
  - Blind points of observation
  - Lack of information on pressure change

pharyngeal residue are impaired pharyngeal bolus transport and imperfect upper esophageal sphincter (UES) relaxation.[8] The former cause was demonstrated in our study by the coexisting high frequency of poor pharyngeal constriction (82.8%). However, it could not thoroughly explain the whole picture observed in our study, because 5 patients showing pharyngeal retention had fair pharyngeal constriction, and the correlation between pharyngeal constriction and pharyngeal retention was not statistically significant in our study (Table 2). Although Alexander et al[9] demonstrated radiographically normal cricopharyngeal function in all of their 9 irradiated patients, we propose aberrant UES function as another factor leading to late aspiration in these patients. Dysfunction of the UES may be due to relaxation failure, decreased compliance caused by fibrosis, or failed coordination with pharyngeal constriction. If intraluminal manometry can validate the upper esophageal resistance as the major cause of pharyngeal retention, it will then be possible for some NPC patients to benefit from the procedure of cricopharyngeal myotomy. Besides, all 9 of our patients with vocal cord palsy showed aspiration during swallowing, no matter how the other defense mechanisms behaved. This result implies that intact vocal cord function may be a prerequisite for protecting the airway from aspiration.

Two major morbidities of these dysphagic patients were repeated aspiration pneumonia and progressive body weight loss, which were final effects of these anatomic and functional impairments. Clinically compromised patients in our study were subject to higher rates of each functional and anatomic impairment, except for dry mouth and velopharyngeal incompetence. Every compromised patient suffered from postswallow aspiration and revealed pharyngeal retention on FEES. The lack of statistical significance was probably due to the small sample sizes. However, no single impairment or cluster of impairments was found to be critical to the final clinical outcome of these irradiated NPC patients. Multiplicity was rather a determinant. The more items of deglutition dysfunction and anatomic changes were involved, the more likely a patient was to become clinically compromised. In addition, there was no correlation between the irradiation dosages and clinical results (Table 1).

Multiple impairments of pharyngeal functions, as shown in Table 2, were a feature of these irradiated NPC patients. A significant correlation was found between aspiration during swallowing and vocal cord palsy. Both poor pharyngeal constriction and pharyngeal retention were correlated with postswallow aspiration. These findings were in accordance with the aforementioned pathophysiology of deglutition disorders. Other significant relationships were found between an abnormal swallow reflex and velopharyngeal incompetence, as well as poor pharyngeal constriction. A possible reason is the involvement of related neuromuscular mechanisms.

After the possibilities of local recurrence and spinal cord metastasis were ruled out, these pharyngeal dysfunctions and anatomic changes appeared to be the result of postirradiation effects, including soft tissue fibrosis, neuropathy, and probable myelopathy.[10-13] Though a linear time-dose relationship was demonstrated by Berger and Bataini[10] in their 35 instances of radiation-induced cranial neuropathy, the radiation dosages in our subjects showed no correlation with the latency interval before onset of significant dysphagic symptoms or with the severity of the pharyngeal dysfunctions. Anatomic changes such as tongue atrophy and vocal cord palsy were also not related to the dosage of radiation. Individual differences in sensitivity to radiation might therefore play a major role in the occurrence of deglutition disorders. Studies on patients who received even higher dosages due to recurrence are needed to clarify the dose effect of irradiation. Impaired pharyngeal function was most often bilateral and not consistent with the original side of neck metastasis in our study. This finding implies that radiotherapy might more often cause diffuse dysfunction than unilateral injury. Both sides of the neck were usually exposed to irradiation.

This is a preliminary study of using FEES to assess deglutition dysfunction and to speculate on the possible pathophysiology in dysphagic NPC patients. The merits of FEES in comparison with videofluoroscopy (Table 3) have been thoroughly discussed in the literature[4,5,14] and our former report.[7] It serves as an easily available and relatively safe method for deglutition evaluation. It is sensitive and efficient for either bedside or outpatient assessment. The disorders detected with FEES are readily recognized, and the results need no time-consuming analysis. It provides the otolaryngologist a quick reference for swallowing safety and a possible regimen, either surgical or physical. After using FEES as the primary tool to assess the pathophysiology of dysphagia in the present study, we found it was possible to use FEES in the identification of major features of swallowing disorders in irradiated NPC patients. This would be a good indication to expand the use of FEES in not only the screening of swallow safety, but also the dynamic physiology study of swallowing disorders. However, quantification of functional deficits is not achievable with FEES, for example, the time sequence of pharyngeal contraction and the pressure changes along the upper digestive tract that occur during swallowing. Manometric studies may augment FEES in the quantification of these functional deficits, especially in clarifying UES dysfunction in the dysphagic patients studied here.

To sum up, in this case series study of NPC patients with dysphagia existing years after irradiation, we found the following.

1. The most common deglutition dysfunction in these patients was pharyngeal retention, which could not be explained solely by the impaired pharyngeal transport mechanism.
2. Intact vocal cord function was the prerequisite for not aspirating.
3. Multiple dysfunctions were a feature of these patients. No single dysfunction was critical in determining the severity of this disorder.
4. Initial staging and irradiation dosage to the neck had no relationship with the development and severity of these deglutition dysfunctions.
5. For assessing pharyngeal deglutition dysfunction, FEES was a handy and useful tool.

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OSTEOMYELITIS OF THE SKULL BASE WITH ATYPICAL ONSET AND EVOLUTION

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Skull base osteomyelitis arises as a complication of malignant external otitis, but it can be also due to middle ear and/or mastoid infection. Other causes can be infections of the paranasal sinuses or of the mandible or maxilla due to odontic caries. Generally, osteomyelitis involves elderly patients affected by diabetic immunodeficiency or microvascular disease. In this paper, we present 3 new cases of skull base osteomyelitis with atypical onset and evolution. The difficulties of diagnosis and details of the management are discussed.

KEY WORDS — complications, osteomyelitis, skull base.

INTRODUCTION

Skull base osteomyelitis may be a complication of malignant external otitis or middle ear and/or mastoid infection. Infections of the paranasal sinuses or of the mandible or maxilla due to odontic caries can also cause skull base osteomyelitis. Generally, it involves elderly patients affected by diabetic immunodeficiency or microvascular disease.[1,2]

A review of the literature showed that certain cases differed from the traditional clinical presentation, previously summarized,[3,4] by presenting with an atypical onset and evolution. Sie et al[5] reported 2 cases of unknown cause, in which predisposing factors or infectious foci could not be detected. Ohashi et al[6] also documented a patient with no predisposing factors and an unusual sign characterized by the presence of a slow-growing tumor in the postauricular sulcus.

The aim of this study was to present 3 new cases of skull base osteomyelitis that exhibited an atypical onset and evolution, with an emphasis on diagnostic evaluation and different modalities of treatment.

CASE REPORTS

Case 1. A 75-year-old man had a 2-month history of progressive swallowing disorders, right unilateral hearing loss, and progressive headache. At otolaryngological fibroscopy, right vocal cord palsy and right-sided movements of the uvula and soft palate were documented. This was compatible with a syndrome of the jugular foramen (Vernet's syndrome). Otoscopic findings were normal. His audiogram demonstrated a 60-dB right conductive hearing loss in the frequencies between 250 and 4,000 Hz. He had also a slight left conductive hearing loss of 30 dB. Blood tests revealed a moderate leukocytosis (9,300 cells per milliliter) and a high erythrocyte sedimentation rate (ESR; 95 mm/h). All other parameters had normal values. The plasma glucose concentration was 100 mg/dL (normal 85 to 110 mg/dL), and there were no typical diabetes mellitus symptoms (polyuria, polydipsia, ketonuria). A lack of immunodeficiency was shown by normal immunoglobulin concentrations and T-cell mediated immunity (T-cell and T-cell subset enumeration). A computed tomography (CT) scan was performed. The images showed a large soft tissue mass occupying the right jugular foramen with areas of bone erosion. Also, osteolytic lesions were present in the occipital bone (Fig 1).

The clinical picture was suggestive of a malignant tumor, and an excisional biopsy was planned. A postauricular approach did not reveal local mastoiditis; rather, bony necrosis was documented, with a moderate sequestrum at the level of the sigmoid sinus. A biopsy specimen was sent for histopathologic and microbiological evaluation. Histopathology disclosed a chronic inflammatory reaction with areas of active disease. *Pseudomonas aeruginosa* developed in culture.

The clinical data were suggestive of osteomyelitis of the skull base. Intravenous therapy with imipenem was begun immediately and was continued for 1 month. Then, oral treatment with ciprofloxacin hydrochloride was administered for 3 weeks. Over a period of 30 days under medical treatment, the ESR and the white blood cell count progressively returned to normal values. The symptoms due to cranial nerve involvement did not improve. Antibiotic treatment

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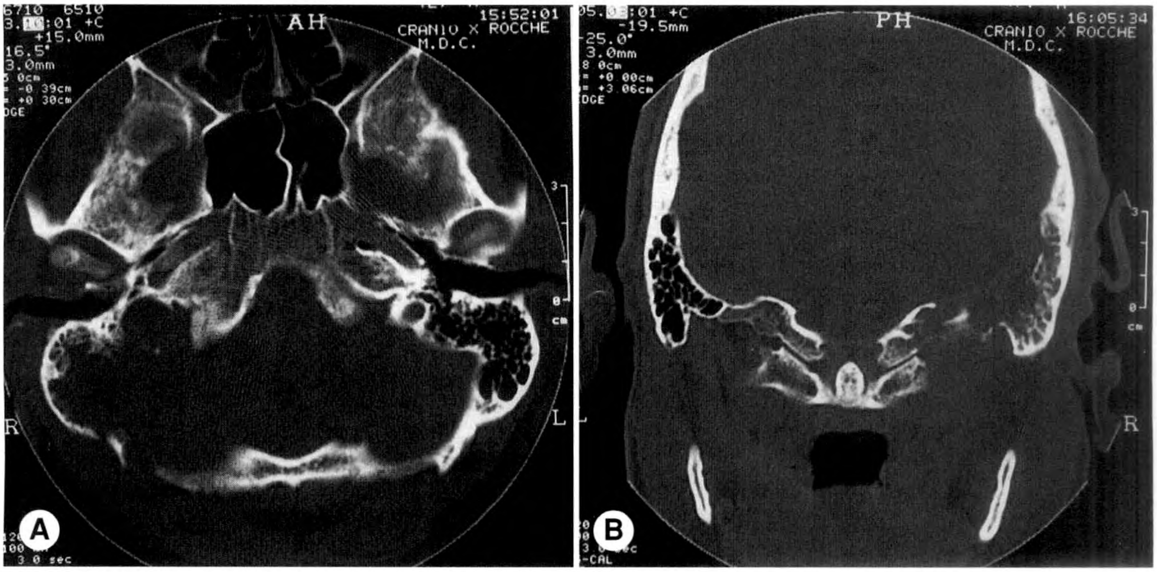

Fig 1. (Case 1) Computed tomography scan. A) Axial view. Right temporal and occipital bones show osteomyelitic inflammation with bony erosion. B) Coronal view showing right diffuse osteomyelitis of jugular foramen.

was interrupted after persistence of normal laboratory values. A 2-year follow-up did not reveal local recurrence of the disease.

Case 2. A 24-year-old man affected by medial fibrosis of the external ear canal (acquired postinflammatory atresia) had been medically treated and followed-up by an otolaryngologist for 3 years. During this period, the patient presented conductive hearing loss and suffered from 2 inflammatory episodes that had been favorably treated with oral antibiotics. Two and a half months before the first visit to our hospital, the patient had complained of an insidious onset of postauricular pain with nightly exacerbations. The patients' history showed no evidence of infectious disease, a traumatic event, or any other condition suggestive of the clinical manifestations. Medical treatment with a nonsteroidal anti-inflammatory drug did not give any considerable improvement. It is interesting to note, however, that the patient had experienced 2 episodes of osteomyelitis of the clavicle of idiopathic origin, and that both his father and his brother had had osteomyelitis of the ulna and of the tibial bone, respectively. In both cases, no specific etiologic factor had been recognized. Ten days before being admitted to the hospital, the patient noted a swelling of the temporal bone region above the external ear canal. Otoscopy confirmed the presence of a external ear canal stenosis, with no evidence of an acute inflammatory infective process.

Hematologic results were in the normal range except for the presence of a high ESR (107 mm/h) and moderate leukocytosis (10,800/mL). The glycemia levels were in the physiological range in every plasma control. The values of serum immunoglobulins (IgA, IgG, IgG subclass, IgM, IgD) were normal, and T-lymphocytes showed normal differentiation and function.

A CT scan revealed an underlying edematous reaction of the mastoid tissue, a lytic lesion of the mastoid cortex at the zygomatic bone root, and diffuse osteitis in the mastoid region (Fig 2). A diagnosis of acute mastoiditis was made, and surgery was planned. It did not detect a purulent exudate, even though an extensive osteomyelitic reaction of the temporal bone could be observed. After multiple biopsies, culture developed *P aeruginosa* colonies. Histologic examination revealed a chronic inflammatory status with isolated areas of active disease. The bone tissue showed extensive necrosis.

Skull base osteomyelitis was diagnosed, and immediate endovenous therapy with imipenem was begun and continued for 8 weeks. After therapy, the ESR had decreased to 15 mm/h, and the white blood cell count had returned to normal values. No recurrence was observed in the following year.

Case 3. A 34-year-old man had had a severe car accident 6 months earlier, sustaining multiple fractures of 4 ribs, the left femur, the condyle, and the right ramus of the mandible. The femoral and mandibular bone fractures were surgically treated at another hospital.

The maxillofacial surgeon observed an extensive involvement of the glenoid cavity. After feeling well for about 20 days, the patient developed, under antibiotic coverage, an insidious onset of postauricular pain with nightly exacerbations. An otolaryngologist was consulted, both a normal external auditory canal

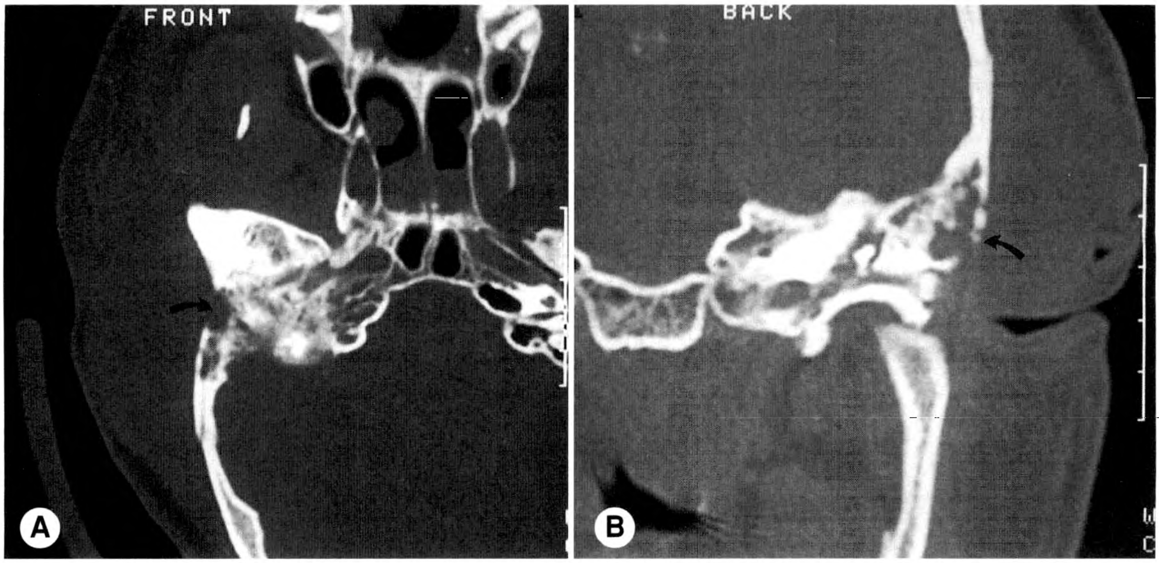

Fig 2. (Case 2) Computed tomography scan. A) Axial view. Arrow indicates osteitic bony erosion of mastoid. There is edematous reaction of subcutaneous tissue. B) Coronal view. Mastoid cortex is eroded (arrow).

and a normal tympanic membrane were documented, and antibiotic therapy was reinstituted.

The pain disappeared after a few days, but 10 days later, a massive bilateral neck lymph node reaction ensued. The lesion developed a phlegmonous inflammatory reaction with purulent cervical drainage. Surgical curettage of the cervical abscess was planned. During surgical resection, culture of the secretions detected the presence of numerous colonies of *Staphylococcus aureus*. Antibiotic therapy with ceftazidime was started. Despite 2 weeks of endovenous therapy, the clinical picture did not improve; rather, a severe deterioration of symptoms with visual disturbance was documented. On the basis of an ophthalmologic examination, a devastating infectious disease of the orbit was diagnosed.

Computed tomography detected a diffuse osteolytic lesion of the facial bones, of the temporal bones bilaterally, and of the sphenoid bone, apparently originating from the mandible fracture.

Failure of antibiotic therapy called for a new surgical curettage of the mandible, of the zygomatic bone, and, unfortunately, of the infected orbit, with the need to remove the eyeball. The cultural examination performed in these circumstances indicated the presence of *P aeruginosa*. Again, imipenem therapy was performed. Unfortunately, no satisfactory results were obtained. Two days later, bilateral purulent otorrhea ensued. Therefore, the patient was transferred to our hospital. Hematology examination disclosed a normal value of plasma glucose (<110 mg/dL), and there was no evidence of immunodepression, given that both the concentration of serum immunoglobulins and the T-cell function were within a normal range. Findings were limited to a high increase in ESR (150 mm/h) and a moderate increase in white blood cell count (12,000/mL).

A CT scan confirmed an extensive osteomyelitic reaction of both temporal bones. Surgical removal of the infective foci at the mastoid level was performed (Fig 3A,B).

Histologic examination of the biopsy specimens taken during surgery confirmed the presence of bone sequestration. The extensive osteomyelitis required a bilateral infralabyrinthine approach (Fig 3C).

The purulent drainage in the cervical region stopped after surgical treatment. Antibiotic therapy with ciprofloxacin and imipenem for 6 weeks, associated with hyperbaric therapy, finally brought total eradication of the inflammatory infectious process. Six months after medical therapy, no recurrence has been documented.

DISCUSSION

This small series consists of 3 new cases of osteomyelitis of the skull base that showed an atypical onset and evolution. The analysis of the clinical course discloses the possible variants of this aggressive, progressive, and potentially devastating disease.

The first feature to notice, common to all 3 patients, is the absence of diabetic or other immunodeficiency or any other diseases that usually are considered to be predisposing factors in the development of osteomyelitis.[1,2]

The first patient exhibited a course characterized by absence of clear infectious processes or other preexistent vasculopathies. The patient did not complain

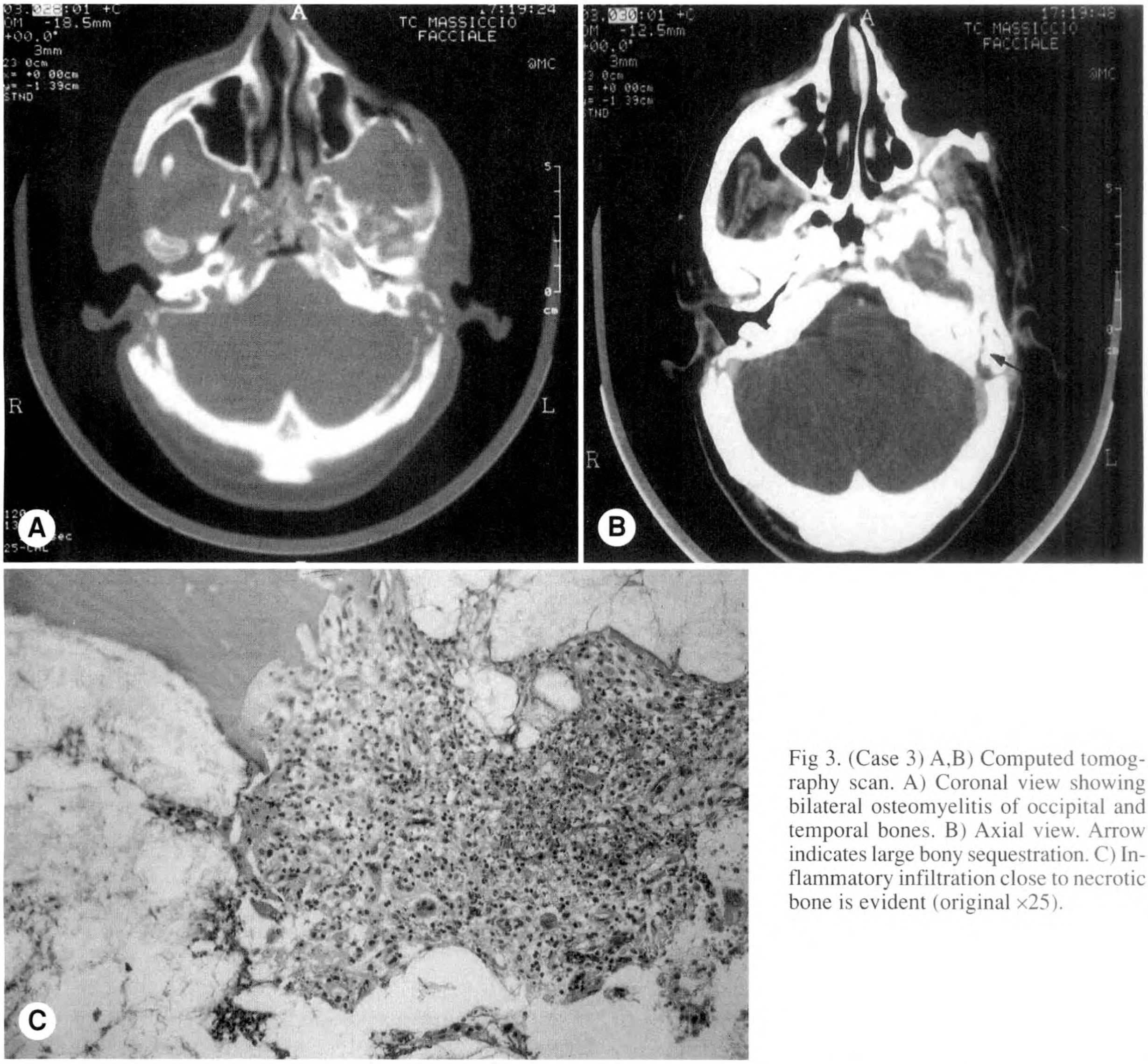

Fig 3. (Case 3) A,B) Computed tomography scan. A) Coronal view showing bilateral osteomyelitis of occipital and temporal bones. B) Axial view. Arrow indicates large bony sequestration. C) Inflammatory infiltration close to necrotic bone is evident (original ×25).

of any initial symptoms, but rather, exhibited a clinical picture of jugular foramen syndrome. A similar case has been documented by Sie et al.[5] In contrast to this latter, a malignant tumor of the temporal bone was suspected as the initial diagnosis — also for the diffuse osteolytic areas in the cranial bones that were seen at CT.

Histologic and culture examination, according to the histologic criteria categorized by Waldvogel et al[7] to define skull base osteomyelitis (inflammatory osteitis, pathogen isolation, and specific imaging alterations), addressed the correct diagnosis.

An initial erroneous evaluation had been performed in our second patient, also. The relatively rapid growth of the postauricular tumor caused it to be diagnosed as acute mastoiditis. The absence of purulent exudate induced us to suspect an osteomyelitis. Moreover, it is important to point out that both the patient and his relatives (father and 1 brother) had previous episodes of osteomyelitis involving other bone structures, leading us to hypothesize a tendency to vascular disorders.[5]

In the third patient, diagnosis was relatively easy, due to the clinical evidence of an initial infectious site. The infectious process arose from the mandible and involved the other facial bones. This progressive involvement evolved with an atypical course (cervical phlegmon with subsequent fistula). The complications were devastating, as they required removal of an eyeball.

It is common knowledge that the diagnosis of osteomyelitis is facilitated by the use of radionuclides. Both gallium and technetium can give pertinent information regarding inflammatory conditions of the soft tissue (mainly gallium) and of the bony structures. However, neither agent is specific for the diagnosis of skull base osteomyelitis.[8-10]

With this in view, both histopathologic examination and detection of the pathogenic organism are essential and irreplaceable. Clinical cases characterized by an atypical onset require a high degree of clinical acumen and thorough consultation before choosing the diagnostic procedures most likely of yield, and embarking on the surgical procedures most likely of success.[5]

Once the diagnosis has been confirmed, antibiotic treatment should be rapidly administered at high doses.[11,12] It is well known that in this disease the most frequent pathogens are *S aureus* and *P aeruginosa*.[13] Our data are analogous to those reported in the literature. Generally, antibiotic administration is performed through an intravenous or intramuscular route. Other efficacious drugs (fluoroquinolones) have been introduced via the oral route.[14,15] Oxygen hyperbaric therapy can be associated to enhance the antibiotic activity, particularly in cases that are resistant or refractory to antibiotic therapy.[16]

A further controversy regards the duration of treatment and its interruption. Besides the clinical parameters of pain control and the resolution of the inflammatory conditions of tissue granulations and discharge of purulent material, different assessments for monitoring the treatment protocol have been recommended. Among those methods, CT and gallium scan must be highlighted. The first method does not give reliable information on the severity of bone involvement. More successful results are obtained with the use of gallium.[5,8-10] This latter substance is universally recommended as a valid indicator of the efficacy of treatment, especially if sequential tests are performed during the various phases of osteomyelitis. In our cases, gallium was not used as a diagnostic tool, because in the first 2 cases, the initial diagnosis was different, whereas, in the third case, the clinical manifestations were markedly evocative and sufficiently defined. For this reason, monitoring of antibiotic treatment was mainly based on the parameters of hematologic examination, particularly the ESR. Rubin and Yu[17] and Johnson and Ramphal[18] have considered it a reproducible test that enables one to monitor antibiotic therapy and dictate its interruption.

A final point must be made about surgical treatment. Generally, it is not recommended, due to the high risk of complications.[5] However, surgical therapy may represent an important adjunctive treatment. In particular, this is true in the presence of coexistent areas of bone sequestration that may constitute an irritative "thorn" and cause persistent infections. Resolution of the osteomyelitis symptoms in the third patient — who did not derive benefit from antibiotic therapy, even though it had been extended for a long period of time — may be paradigmatic of this assumption. Surgery with the removal of necrotic bony tissue facilitated the antibiotic sterilizing action. A similar case has been discussed by Ohashi et al.[6]

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TRIGEMINAL TROPHIC SYNDROME MIMICKING WEGENER'S GRANULOMATOSIS

A CASE REPORT WITH A REVIEW OF THE LITERATURE

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Trigeminal neuropathy with nasal ulceration, called trigeminal trophic syndrome, is an unusual complication of anesthesia in the trigeminal area. We present a case to illustrate the diagnostic and management problems that this syndrome presents.

KEY WORDS — trigeminal neuralgia, trigeminal trophic syndrome.

INTRODUCTION

Trigeminal trophic syndrome (TTS) is a rare and distinct clinical entity resulting from trigeminal nerve damage. Clinical manifestations of TTS may mimic a myriad of pathologically aggressive midfacial diseases, including malignant neoplasms, granulomatous infections, deep fungal infections, lethal midline granuloma, and Wegener's granulomatosis. The majority of patients with this syndrome are elderly women. Trigeminal trophic syndrome is a diagnosis of exclusion based on clinical history and negative pathological findings for malignancy, granulomatous disease, vasculitis, or infection.

We present the case of a patient with TTS who was unsuccessfully treated elsewhere for Wegener's granulomatosis. The patient's clinical presentation of trigeminal anesthesia, facial paresthesias, and deep crescent-shaped ulcer of the lateral ala nasi, and histopathologic absence on biopsy of tumor, vasculitis, or infection, led to the final diagnosis of TTS.

CASE REPORT

A 75-year-old woman was referred to our departments of dermatology and head and neck surgery for evaluation of a persistent ulcer of 2 years' duration on the right side of her face. Her medical history was significant for an acoustic neuroma surgery done in 1980 and a secondary right facial nerve paralysis. She had undergone subsequent surgeries to correct the right facial paralysis and third cranial nerve palsy. Though the patient denied any history of trauma to the area, family members had witnessed the patient scratching the ulcer. However, no one could verify whether the self-induced trauma preceded the ulcer formation. Multiple outpatient biopsies had been previously performed that revealed only nonspecific chronic inflammation with necrotic tissue. Computed tomography of the nose and sinuses revealed mucosal thickening and proliferation in the right paranasal sinuses. There was no evidence of bony involvement. A presumed diagnosis of Wegener's granuloma had been made, and a course of prednisone and methotrexate sodium had been initiated without resolution of the ulcer.

On physical examination, there were no symptoms of systemic illness. Head and neck examinations revealed extensive erythema with ulceration of the right cheek of 4 × 2 cm. Fistula formation at the ulcer base led from the right nasolabial fold to the entire nasal vestibule and maxillary sinus (see Figure). A tarsorrhaphy was done for ocular protection because of right persistent facial nerve weakness. There was sensory loss to light touch on the right side of the face, including the forehead. The remainder of the findings on head and neck examination were unremarkable. Her general physical examination findings were only significant for the presence of an unsteady gate.

The patient was admitted to the hospital, and multiple intraoperative biopsies were performed. The histopathologic findings showed granulation tissue and inflammation with no evidence of a neoplastic, infectious, or autoimmune process. All bacterial, fungal, and mycobacterial cultures were negative. A diagnosis of TTS was made on the basis of the history and

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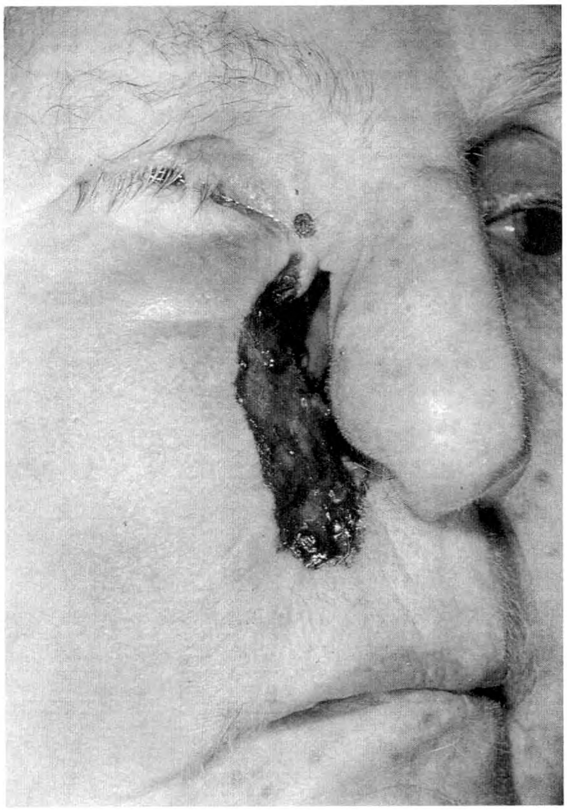

Frontal view of patient, showing crescent-shaped lesion.

clinical and pathological findings. Local wound care was instituted, and psychiatric consultation obtained. A trial of transcutaneous electrical stimulation produced significant discomfort in the patient and was shortly discontinued. A short-term treatment with Paxil was also not tolerated. The patient refused further management, including reconstruction of the defect with a vascularized flap.

DISCUSSION

Trigeminal trophic syndrome was first described in 1933 by Loveman,[1] who termed it "ulceration en arc." The clinical presentation comprises the triad of trigeminal anesthesia, altered facial sensation (paresthesia), and crescent-shaped lateral nasal ala erosion or ulceration.[2]

Trigeminal anesthesia can develop after any infectious, traumatic, autoimmune, iatrogenic, degenerative, or vascular condition that damages the sensory root of the trigeminal nerve in the gasserian ganglion. Most cases of TTS occur after surgical or chemical intervention for trigeminal neuralgia. Less frequent causes of this syndrome are vertebral basilar insufficiency (9%), acoustic neuroma (5%), postencephalitic parkinsonism (5%), syringobulbia (2%), and idiopathic causes (5%).[3] The period between the onset of trigeminal sensory deficit and the appearance of TTS ranges from 2 weeks to 18 years, with a median interval of 1 year.[3]

The clinical onset of paresthesia is believed to be due to the innervation differences between the sensory portions of the 3 divisions within the trigeminal nerve (ophthalmic, maxillary, and mandibular), extending from the ear to the anterior facial midline. Typically, the ulcers in TTS present in the area of sensory overlap between the first and second divisions of the nerve (ophthalmic and maxillary).[2]

Although self-induced trauma is the primary cause of this syndrome, there is a proposed pathophysiological mechanism predisposing patients to the development of ulcer formation. It is hypothesized that destruction of the gasserian ganglion results in autonomic vasomotor dysfunction, as sympathetic fibers from the internal carotid plexus to the trigeminal ganglion are disrupted. The resultant low sympathetic tone leads to constant cooling of the skin secondary to vasodilatation and slower venous return. This results in an unfavorable condition for metabolic processes involved in wound healing, augmenting the potential for nonhealing ulcer formation after trauma.[4] The tip of the nose is often spared because of its innervation by the ethmoidal branch (ophthalmic division V-1).[3]

Other clinical manifestations of this denervation are depigmentation, dermatitis, temporal alopecia, and dry, atrophic nasal mucosa.[1] Patients with TTS often report symptoms of burning, pruritis, and nasal occlusion. These altered facial and rhinological sensations precipitate the self-induced midface trauma.[5]

Trigeminal trophic syndrome clinically mimics many midface neoplastic (basal and squamous cell carcinoma), autoimmune, and infectious processes.[6] Our patient had received an incorrect diagnosis of Wegener's granulomatosis and was treated with both prednisone and methotrexate without clinical response. The periadventitial vasculitis seen in Wegener's granulomatosis is absent in the tissue biopsy specimens of TTS. Similarly, Wegener's granulomatosis often has systemic manifestations of disease, in particular, renal (glomerulonephritis) and pulmonary (multiple parenchymal lesions) involvement.[7] Misdiagnosis of TTS underscores the importance of obtaining an accurate medical-surgical history and the necessity of tissue procurement and careful pathological assessment in the treatment of all midfacial lesions.

Multiple infectious agents can also cause midline ulcerative processes. These include *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Treponema pallidum*, and various fungi. Establishing these agents as the cause necessitates bacterial and fungal cultures with histopathologic analysis of the tissue.[6]

Treatment of TTS is multidisciplinary: recognition of the disease, psychological counseling, and surgical repair by local or regional flaps are the primary methods of treatment.[8] Surgical repair alone, without psychological counseling combined with a clear patient and family awareness regarding the cause of this disease, is less likely to succeed.[9]

Trigeminal trophic syndrome is rare, presenting with the classic triad of trigeminal anesthesia, facial paresthesia, and a crescentic defect of the ala nasi. Awareness of this syndrome increases the likelihood of early recognition and proper management.

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TRANSVERSE LAG SCREW FIXATION IN MIDLINE MANDIBULOTOMY

A CASE SERIES

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Vertical midline mandibulotomy has provided a relatively simple and efficient means of obtaining access to intraoral tumors that are too large or too posterior to be removed transorally. Midline mandibulotomy has had the advantage of nerve and muscle preservation and places the osteotomy outside the typical field of radiotherapy, in contrast to lateral and paramedian osteotomies. Plate and screw fixation has been the usual means of osteosynthesis for these mandibulotomies; however, plate contouring over the symphyseal surface has been a time-consuming process. Unless the plate was contoured exactly, mandibular malalignment and malocclusion in dentulous patients has occurred. Use of parallel transverse lag screws has become a popular method of osteosynthesis for parasymphyseal fractures, and we have extended their use for mandibulotomy fixation. This paper reports our clinical experience with transverse lag screw fixation of midline mandibulotomies in 9 patients from 1994 to 1997. There were 7 men and 2 women with a mean age of 56 (range 35 to 71 years). The pathological diagnosis in all patients was squamous cell carcinoma; 8 cases were primary, and 1 patient presented with recurrent tumor. No tumors involved the mandibular periosteum. One patient had had previous radiotherapy, and 3 patients underwent postoperative radiotherapy. The mean follow-up has been 17 months (range 9 to 27). There was 1 minor complication and 1 major complication related to our technique. The major complication was a delayed nonunion of the mandibulotomy. This occurred because the 2 parallel screws were placed too close to one another, and this placement resulted in a delayed sagittal fracture of the anterior cortex and subsequent nonunion. Transverse lag screw fixation has not affected occlusion in our dentulous patients. Speech and diet were normal in the majority of our patients. Transverse lag screw fixation of the midline mandibulotomy has been a relatively safe, rapid, and reliable method for tumor access and postextirpation mandibular stabilization and has significant advantages over other current methods of mandibulotomy and fixation.

KEY WORDS — lag screws, mandibulotomy, oropharyngeal carcinoma.

INTRODUCTION

Midline mandibulotomy was first performed by Roux in 1936 to approach intraoral tumors that were too large or too posterior to be removed transorally. In 1944, Sedillot modified Roux's vertical mandibulotomy by creating an angular osteotomy so as to better reapproximate the mandibular segments. Postoperative stabilization in these techniques was provided by a gold plate secured to the teeth with silk sutures. As Bilroth in 1962 developed a modified tumor resection, and radical tumor excision with an adjacent segment of mandible became popular, mandibulotomy fell into disfavor.[1] Composite resection of the mandible with tumor excision assured the surgeon of wide field exposure, safe margins, and improved closure, with or without mandibular tumor invasion.[2] Since Marchetta et al[3] suggested in 1971 that partial mandibulectomy for oral tumors was not necessary when periosteal or frank bony invasion was not evident, there has been a resurgence of interest in preserving the mandible. As more centers have used mandibulotomies and documented their utility and low morbidity,[4-6] variations in osteotomy and fixation techniques have been developed.

New methods of mandibulotomy fixation often have paralleled developments in the treatment of mandibular fractures. Direct wiring of the mandibular segments[5] and wiring supplemented with a transfixing Kirschner wire[7] have been supplanted by rigid fixation with compression plates or miniplates,[4,8] and nonunion has since been uncommon. Transverse lag screws have been used successfully in the fixation of mandibular symphyseal fractures.[9] By applying this technique to the reestablishment of mandibular continuity after mandibulotomy, rigid fixation and normocclusion have been achieved, while avoiding the need for plate contouring over the curved and angled surface of the symphysis, which has been a time-consuming process.

Two reports have described a means of achieving rigid mandibular fixation while minimizing the amount of hardware present under the gingivolabial suture line.[10,11] These procedures used the technique

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PATIENT DEMOGRAPHICS AND OUTCOME

Pt No.	*Sex*	*Age*	*Date of Surgery*	*Dentulous at Surgery*	*Tumor Type and Location*	*Stage*	*Operating Room Time**	*Follow-up (mo)*	*Outcome*
1	M	62	9/94	Yes	Squamous cell, base of tongue	T2N0M0	8 h 41 min	27	Required secondary procedure, mandible stable, NED 4/97
2	M	69	4/95	No	Squamous cell, base of tongue to tonsillar fossa	T2N1M0	8 h 39 min	31	NED 5/96
3	M	56	9/95	No	Recurrent squmous cell, base of tongue to tonsillar fossa	T2N1M0	6 h 21 min	21	DOD 5/96
4	M	71	4/96	No	Squamous cell, floor of mouth	T2N0M0	8 h 43 min	11	DOD 3/97
5	F	50	6/96	Yes	Squamous cell, base of tongue	T3N2M0	8 h 31 min	16	LWD 2/97
6	M	43	7/96	No	Squamous cell, base of tongue and palate	T2N0M0	8 h 16 min	16	NED 6/97
7	M	48	9/96	Yes	Squamous cell, base of tongue	T3N2M0	10 h 50 min	14	NED 6/97
8	M	69	12/96	Yes	Squamous cell, base of tongue	T2N1M0	9 h 9 min	11	NED 6/97
9	F	35	2/97	Yes	Squamous cell, base of tongue	T3N2M0	7 h 39 min	9	NED 6/97

NED — no evidence of disease, DOD — dead of disease, LWD — living with disease.

*Operating room time, including extirpation and reconstruction.

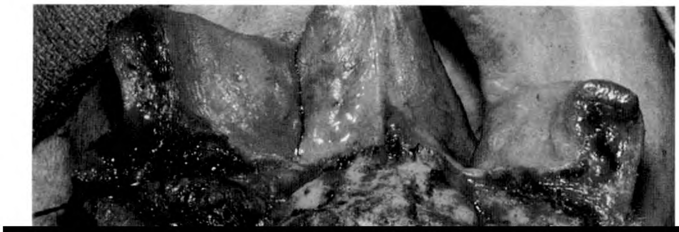
of lag screw fixation but required complex stepped buccal and lingual corticotomies. While these techniques avoided plate contouring, the complex osteotomies remained a time-consuming process. We previously introduced a rapid technique using lag screws to fixate the traditional straight midline mandibulotomy.[1] This report updates our results with this technique on the basis of our increased clinical experience, including use of the technique in dentulous patients.

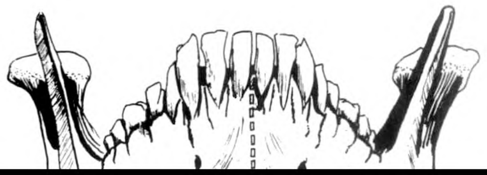
PATIENTS AND METHODS

Beginning in 1994, all patients referred to our center with an oral cavity or oropharyngeal carcinoma requiring mandibulotomy for tumor access and reconstruction were considered candidates for midline mandibulotomy and transverse lag screw osteosynthesis. Hospital and office records were reviewed for patient demographics, date of surgery, dentate status, tumor type and location, stage, operating room time, complications, and patient outcome (see Table).

TECHNIQUE

A midline lip-splitting incision was performed to expose the mandibular symphysis. The periosteum





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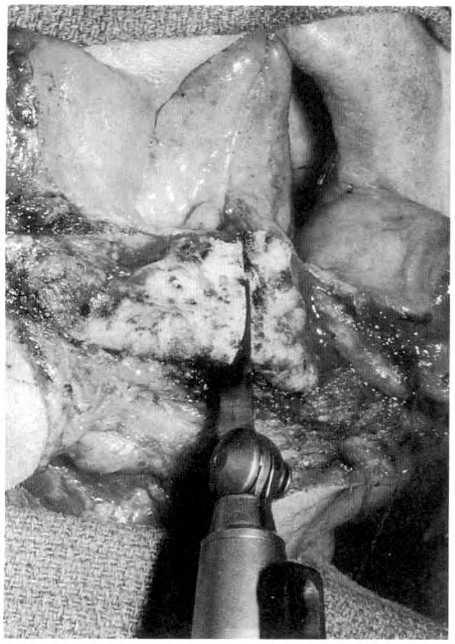

Fig 2. Midline mandibulotomy is made with thin blade on sagittal saw. Reprinted with permission.[1]

was elevated bilaterally toward the mental foramina. Two parallel 1.8-mm drill holes were then made transversely across the mandibular symphysis anterior to the mental foramina (Fig 1[1]). Each hole was irrigated with saline to remove any bony debris remaining from the drilling process. A measuring probe was inserted into the drill hole to determine screw length, which was recorded for later screw placement. A straight vertical midline osteotomy was then performed with a thin sagittal saw blade (Fig 2). In dentulous patients, the saw cut ended just inferior to the alveolar bone, between the central incisors. A sharp, fine osteotome was used here to complete the osteotomy and minimize injury to the blood supply to the incisors. The attachments of the submental muscles were preserved. Once the mandible was divided, the drill hole on 1 side of the mandibulotomy became the gliding hole for the lag screw technique. This was accomplished by overdrilling the previously placed drill hole with a 2.4-mm drill bit (Fig 3[1]). Overdrilling once the mandibulotomy was completed allowed direct visual control, ensuring that the gliding hole remained on only half of the osteotomy. Upon completion of the tumor extirpation and reconstruction, the 2 halves of the mandible were manually reapproximated. Self-tapping 2.4-mm screws (whose lengths were measured previously and recorded) were then inserted through the gliding hole and into the traction hole, establishing rigid fixation (Fig 4[1]). The surgery was then completed with soft tissue closure. Location of the lag screws was then confirmed radiographically (Fig 5[1]). The patient resumed his or her diet once the mucosal incisions were healed.

RESULTS

Since 1994 our center has treated 314 patients with head and neck malignancies. In that group of patients, 9 were selected before surgery as requiring a mandibulotomy for tumor extirpation and reconstruction. All 9 were treated with a midline mandibulotomy and lag screw osteosynthesis. There were no alterations in our technique as described above for any of the 9 patients. No patient was converted to a mandibulectomy. No form of fixation other than the 2 lag screws was used in any patient.

There were 7 men and 2 women with a mean age of 56 (range, 35 to 71). Eight of the tumors were primary squamous cell carcinomas predominantly located at the base of the tongue. One tumor was a squamous cell carcinoma that had recurred after previous resection 5 months earlier. Two of the tumors extended to involve the ipsilateral tonsillar fossa, and 1 extended to the palate. One tumor predominantly involved the floor of the oral cavity. None of the tumors involved the mandibular periosteum. Six of the 9 patients had stage II disease, and 3 patients had stage III disease. Three patients underwent postop-

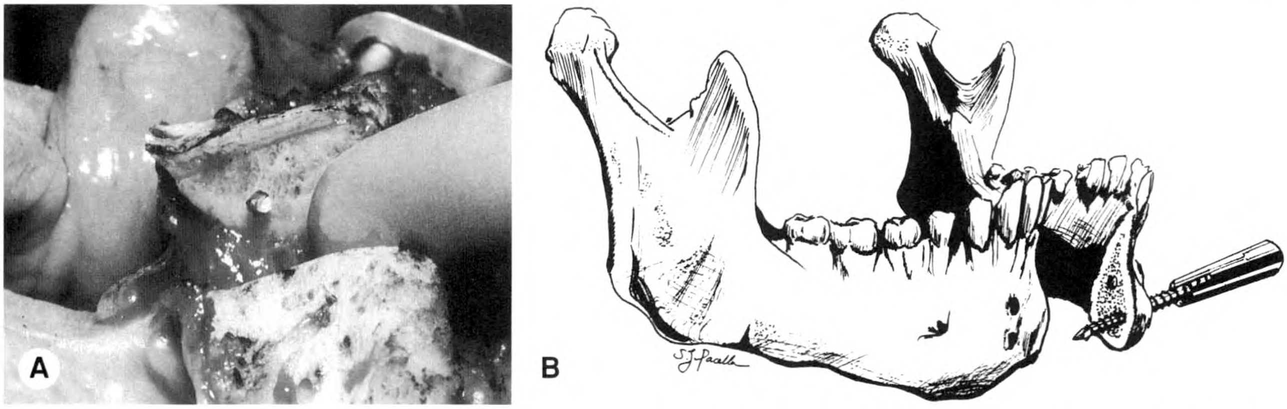

Fig 3. After completion of saw cut, gliding holes are created by overdrilling original drill holes on 1 side of mandibulotomy with 2.4-mm drill bit. A) Reprinted with permission.[1] B) Diagram.

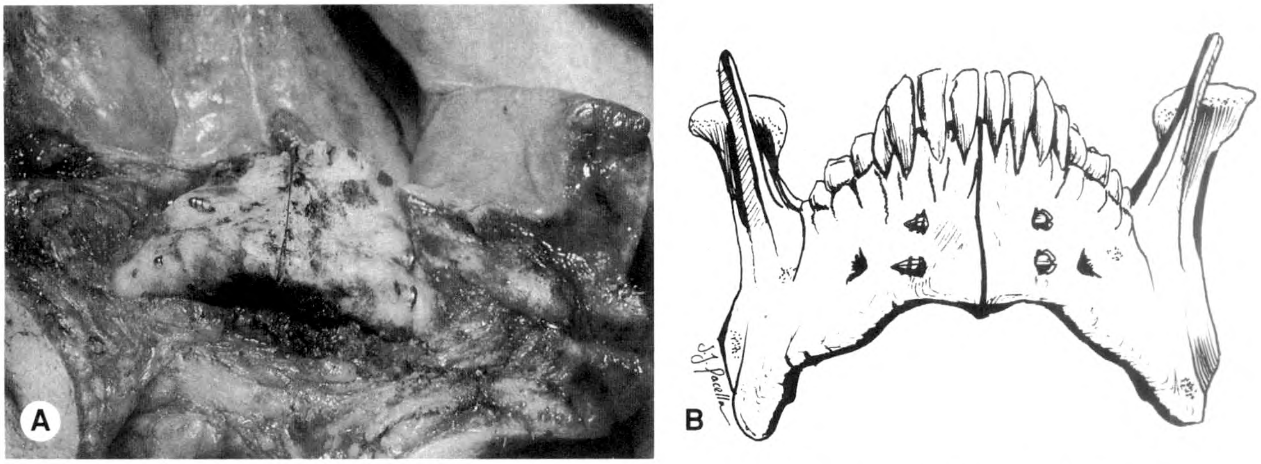

Fig 4. After completion of extirpation and reconstruction, mandibular segments are reapproximated and rigidly fixed with two 2.4-mm screws. Screws are measured for length and inserted into previously drilled holes to produce anatomic mandibular alignment. A) Reprinted with permission.[1] B) Diagram.

erative radiotherapy, and 1 had had radiotherapy previously.

The mean surgical time for extirpation and reconstruction was 8.5 hours (range, 6.3 to 11.8). The mean follow-up of our patients has been 17 months (range, 9 to 27). Six patients (67%) were able to return to a regular diet, all within 3 months after surgery. Three patients (33%) have maintained a soft or puree diet. All patients underwent postoperative speech therapy, with 8 patients (89%) returning to normal speech. Two patients (22%) reported significant drooling and/or pooled saliva in the oral cavity.

Two of our patients developed late problems requiring operative intervention. One patient developed pain at the osteotomy site, with a small area of anterior mandibular exposure 3 months after surgery. This appeared to be a long-standing small dehiscence related to the sharp edges of the edentulous alveolar bone on either side of the osteotomy site. This area of bone was debrided, and the soft tissues were reapproximated. The intraoral areas healed; however, the patient continued to experience pain at the osteotomy site. The osteotomy remained stable on manual examination. This patient was eventually found to have inoperable metastases and was referred to hospice care 6 months after surgery. He died 3 months later.

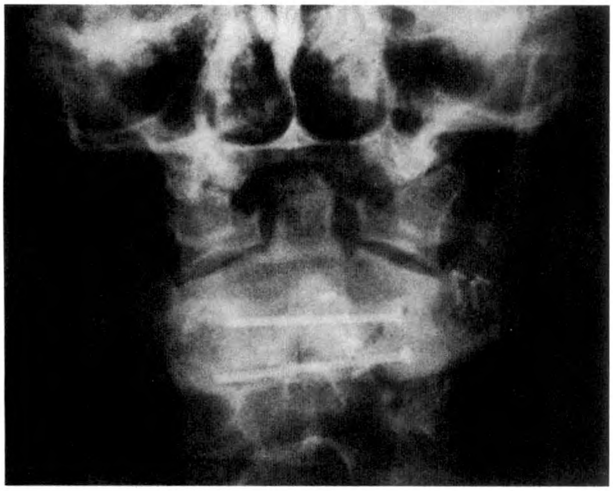

Fig 5. Postoperative radiograph shows parallel screw placement and healing osteotomy site. (Reprinted with permission.[1])

A second patient had an unremarkable postoperative course until 12 months, when he reported some purulent drainage from the submental skin. Bone scan, computed tomography, biopsy, and microbial culture subsequently confirmed fibrous nonunion of the mandible with a segment of necrotic bone. The patient was taken to the operating room for debridement. A sagittal fracture of the right side of the anterior mandible, just anterior to 1 of the lag screw holes, was discovered. This sagittal fragment was necrotic and was causing the purulent drainage. The areas of nonunion and necrotic bone were debrided, and a 2.4-mm reconstruction plate was placed to bridge a small mandibular defect. After allowing for healing and wound maturation, we returned the patient to the operating room for placement of a nonvascularized iliac crest bone graft through an extraoral approach. This patient has maintained mandibular stability, but did develop a malocclusion. The patient has denied any problems related to the malocclusion and has not been interested in any therapeutic intervention to treat it.

Five of our patients were dentulous. All 5 of these patients reported normal occlusion after surgery. Four of these 5 patients have maintained normal occlusion. The 1 patient described above developed a malocclusion after resection of the area of nonunion and stabilization with a reconstruction plate.

DISCUSSION

Mandibulotomy for oral cavity and oropharyngeal extirpation has provided an excellent means of intraoperative exposure for both resection and reconstruction of selected tumors.[5] Variations on the mandibulotomy technique have included a stair-step osteotomy, a parasymphyseal osteotomy, and a lateral osteotomy.[12] Other authors have expressed a preference for a lateral or paramedian mandibulotomy.[13,14] The midline vertical osteotomy has been our choice over other techniques, for several reasons. It is simpler and quicker to perform than any stepped osteotomy. The anterior placement of the osteotomy preserves the inferior alveolar, mental, and lingual nerves, as well as the attachments of the submental muscles. In addition, the midline mandibulotomy places an osteotomy in a location that usually does not receive postoperative irradiation.[12] The disadvantage of the midline location is the resulting overlap of the mucosal incision with the osteotomy, with the potential for fistula, infected nonunion, or osteomyelitis of the mandible.

The placement of metallic foreign bodies used for bone stabilization underneath the gingivolabial suture line has further complicated soft tissue stabilization.[15] Gingival closure over a plate predisposes the suture line to tension and thus dehiscence. In addition, contamination of the mandibular plate by saliva increases the risks of these infectious sequelae. The use of the lag screw technique has avoided these problems by placing the metallic foreign body some distance both from the buccal sulcus suture line and from the anterior surface of the mandible. In addition, this technique has also allowed for significant savings of operative time and has limited the risks of postoperative malocclusion by avoiding the need for complex plate contouring over the angled surfaces of the symphysis.

Nonunion was seen in only 1 of our patients, and it presented as a late complication. We believe that this nonunion was a direct result of a technical error made during the drilling process. The parallel holes in this patient were made too close to one another and too anterior, resulting in a sagittal fracture of the anterior cortex of the mandibular symphysis. The nonunion, in combination with the devascularized cortical fragment, led to subsequent submental purulent drainage. The drill holes should be placed as far apart from each other as possible while still remaining in the denser portions of the symphyseal bone and while also avoiding injury to tooth roots in dentulous patients. The drill holes should also be passed through as much of the symphysis as possible, given the bone's width and the maximum screw length of 40 mm.

Complications were limited to 2 of our 9 patients (22%). Only 1 of these patients had a complication directly related to the fixation technique, corresponding to a nonunion rate of 11%. A 35% complication rate has been reported with the use of plate and screw fixation in both the midline and parasymphyseal positions. The most common complications in that series were nonunion (19%) and plate exposure (13%).[15] In another series, wire fixation was associated with a higher complication rate (29%) as compared with plate osteosynthesis (5%).[6] In a large series of paramedian notched osteotomies with wire fixation, 20% of patients had osteotomy-related complications.[14] Techniques using midline osteotomy with plate fixation, midline osteotomy with Kirschner wire fixation, parasymphyseal osteotomy with miniplate fixation, stair-step sagittal osteotomy with lag screw fixation, and stair-step osteotomy with plate fixation have been published as small patient series including their associated complication rates.[4,7,8,10-12] Our technique has compared favorably with the reported complication rates of the other techniques described above and remains an easier and more rapid means of mandibulotomy and fixation.

Malocclusion has been a frequent result of incorrectly applied rigid fixation devices in mandibular fracture treatment.[7] Loss of bone in the anterior mandible from mandibulotomy has the potential for malocclusion. We believe that using a thin saw blade to create the osteotomy creates a very limited loss of anterior bone. Minute alterations in mandibular segments have usually been accommodated by the dynamic nature of teeth within the alveolar bone. We believe that this process, along with the limited anterior bone loss, explains the absence of malocclusion in our dentulous patients.

Transverse lag screw osteosynthesis of the midline mandibulotomy has provided a relatively safe, rapid, and reliable method of mandibulotomy and fixation in the majority of our patients and has significant advantages over other current osteotomy and fixation methods.

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SAN FRANCISCO OTOLOGY & NEUROTOLOGY UPDATE 2000

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IMAGING CASE STUDY OF THE MONTH

MULTIPLE CRANIAL NERVE PALSIES AS A COMPLICATION OF INFECTIOUS MONONUCLEOSIS DUE TO INFLAMMATORY LESION IN JUGULAR FORAMEN

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Infectious mononucleosis (IM), a manifestation of Epstein-Barr virus (EBV) infection, is a common acute viral illness characterized by pharyngotonsillitis, fever, and generalized lymphadenopathy. Its symptoms usually pass within 1 to 3 weeks, and recovery is uneventful. In some cases, however, IM may lead to severe complications such as airway obstruction,[1] meningoencephalitis,[2] and Guillain-Barré syndrome.[3] Our report concerns a 20-year-old man who developed IM with airway obstruction, secondary pneumonia, and unilateral palsies of cranial nerves IX, X, XI, and XII. Multiple cranial nerve palsies are a very uncommon complication of IM, and the pathophysiological basis has not been accounted for.[4] Our report is the first of its kind in the literature to deal with the magnetic resonance imaging (MRI) findings that correlate with the paralysis.

CASE REPORT

A previously healthy 20-year-old man was admitted in June 1997, complaining of fever and severe throat pain that had lasted for 6 days. A throat culture performed on his first day in primary care was negative for β-hemolytic group A streptococci, but a Monospot test was positive. Despite penicillin V therapy for 5 days, the patient's throat pain had worsened.

On examination, the patient looked acutely ill and had a temperature of 38.9°C. He had severe tonsillopharyngitis with bilateral peritonsillar and pharyngeal edema and enlarged cervical lymph nodes. Throat and blood cultures were negative, while the serum EBV immunoglobulin M antibody (Enzygnost) was positive. Other laboratory findings were as follows: hemoglobin 152 g/L (normal 130 to 180), leukocyte count 8.9×10^9/L (4.0 to 10.0), lymphocytes 61% (20% to 45%), platelet count 135×10^9/L (150 to 400), C-reactive protein 68 mg/L (0 to 10), sedimentation rate 19 mm/h (1 to 8), and aspartate aminotransferase 75 U/L (0 to 49).

On the second day, an emergency tonsillectomy was performed to alleviate airway obstruction caused by severe edema in the pharynx. The patient was intubated and monitored in the intensive care unit. His arterial oxygen level was 8.9 kPa (normal 10.0 to 14.6). Postoperative chest radiography showed bilateral pneumonia with atelectasis in the right lower lobe. Bronchoscopic examination revealed bilateral reddish swelling of the bronchial mucosa and purulent secretions. A bacterial culture of the bronchial secretions revealed *Moraxella catarrhalis*. The patient was treated intravenously with dexamethasone sodium phosphate (10 mg ×1) and cefuroxime sodium (1.5 g ×3).

On the fourth postoperative day, the patient was extubated. He was dysphonic. During the next 5 days, his hoarseness increased and the patient complained of difficulties in swallowing. He also had a tendency to aspirate food when eating. On examination, the right vocal cord was immobile in the paramedian position, the gag reflex and palatal motion were absent on the affected side, the tongue deviated to the right on protrusion, and the patient could not raise his right shoulder. No other neurologic abnormalities were noted.

A detailed head and neck contrast-enhanced MRI examination was performed on a 1.5-T unit with special emphasis on the posterior fossa, jugular fossa, and surrounding extracranial structures. Intracrani-

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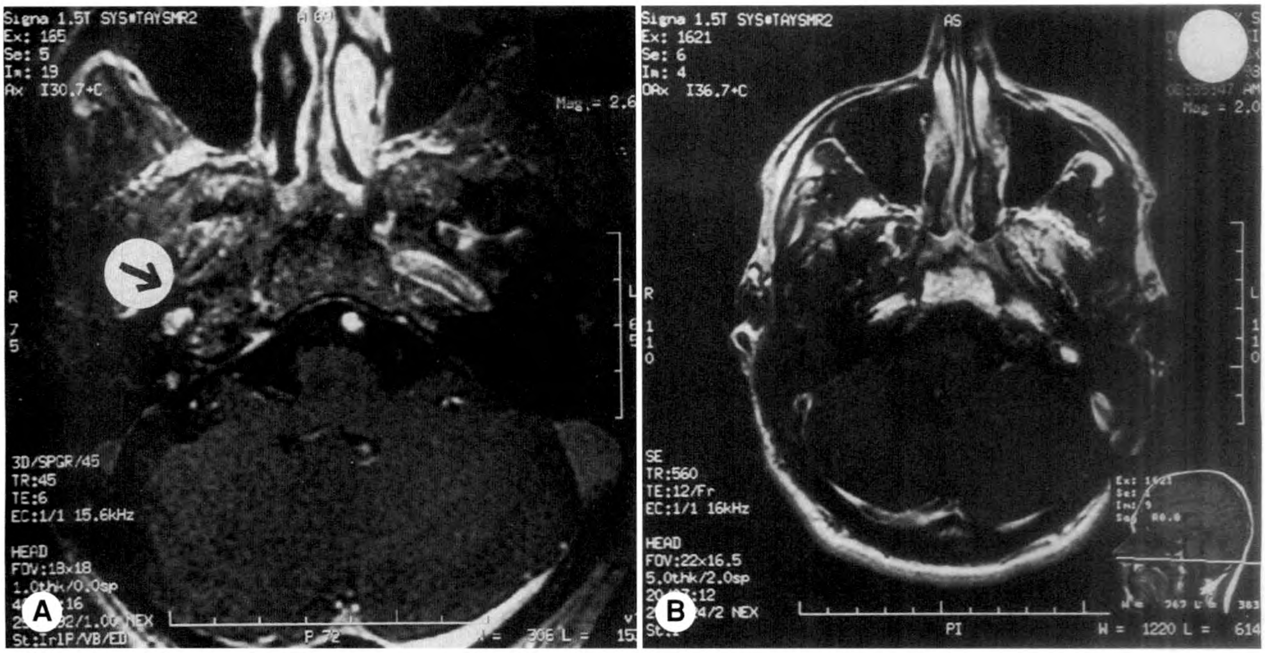

Fig 1. Cranial magnetic resonance images (gadolinium–diethylenetriamine pentaacetic acid–enhanced axial, 1-mm-thin, T1-spoiled GRASS [gradient-recalled acquisition in steady state] sections). A) Revealing expansive, diffuse, nonhomogeneous, [redacted]

U/mL, and the IgM antibody remained positive.

In September 1997, lumbar puncture and electrodiagnostic studies were performed. Cerebrospinal fluid findings included normal cell count, protein content, IgG index, and isoelectric focusing. Electromyography showed fibrillation potentials in the trapezius, sternocleidomastoid, and lingual muscles on the right side. Nerve conduction studies revealed absent accessory nerve motor responses.

The patient received speech therapy and made a complete clinical recovery within 6 months after the beginning of his illness. On control MRI examination, the mass in the right jugular fossa had resolved (Figs 1B and 2B).

DISCUSSION

Neurologic findings can be the sole manifestation of an EBV infection or may accompany the classic clinical picture of IM.[5] The incidence of neurologic complications is generally less than 1%, but among IM patients who need hospital treatment because of the severity of the illness, incidences as high as 5.5% have been reported.[6] Isolated or bilateral cranial nerve palsies have frequently been reported to accompany IM,[7] but a clinical picture of IM with multiple cranial nerve palsies is very rare,[4] and as far as we know, only 1 report has previously been published on this complication with the same cranial nerves involved as in our case.[8]

The pathogenesis of cranial nerve palsies with IM is unclear. Several theories have been proposed, including encephalitis and acute inflammation in the area of the brain stem or midbrain, but there has been no direct evidence to support any of them.[4,9] In our case, a neurologic examination did not show any other abnormalities, and the cerebrospinal fluid examination revealed normal findings. The MRI did not exhibit any findings compatible with meningitis, encephalitis, or any specific cranial nerve inflammation, but did reveal a lesion in the right jugular fossa that resolved on control MRI, thereby indicating its possible inflammatory nature (Figs 1 and 2).

The jugular foramen contains 3 cranial nerves: the glossopharyngeal nerve (IX), the vagus nerve (X), and the spinal accessory nerve (XI). At the medial border of the foramen and to approximately 8 mm inferiorly is the hypoglossal canal, which is traversed by the hypoglossal nerve (XII). In our case, the lesion's being located in this area gave rise to compression of all 4 cranial nerves. Our findings thus suggest that extracranial compression on cranial nerves by inflammatory tissue should be considered as a possible cause for cranial neuropathy in IM patients.

Theoretically, corticosteroid treatment could be beneficial in the prevention and treatment of cranial nerve palsies by alleviating edema in cases of extracranial compression. However, in a double-blind, multicenter study, acyclovir combined with prednisolone treatment was found to be ineffective in relieving symptoms associated with IM.[10] Moreover, our patient received a short course of dexamethasone treatment in the acute phase of his illness, and it did not prevent the manifestation of cranial nerve palsies. In any event, the prognosis seems to be excellent with or without steroid treatment in most cases, with complete recovery taking place within 4 to 6 months.[4,8]

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UPDATE ON THE USE OF AURICULAR CARTILAGE IN LARYNGOTRACHEAL RECONSTRUCTION

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The pediatric otolaryngologist is often called upon to aid in the diagnosis and management of subglottic stenosis. This report contains an update of our experience using auricular cartilage in laryngotracheal reconstruction. A retrospective review of the medical records at St Louis Children's Hospital identified 43 children with subglottic stenosis. Thirty-one children were treated by use of auricular cartilage with a success rate of 84%, and an overall 94% success rate after revision surgery. Eight children in whom an anterior cricoid split initially failed were secondarily treated with auricular cartilage with a success rate of 75%. Two children initially treated with costochondral cartilage underwent multiple reconstructive procedures with either auricular cartilage or costochondral cartilage with an overall success rate of 50%. The remaining 2 children had long-segment tracheal stenosis and underwent repair with auricular cartilage with a 50% success rate. We find that auricular cartilage grafts are highly effective when used in a primary single-stage procedure in children with grade I or II stenosis. We have had limited success with auricular cartilage in patients with grade III stenosis and are reluctant to use it in grade IV stenosis, long-segment tracheal stenosis, staged reconstruction, or revision of an auricular or costal cartilage graft laryngotracheal reconstruction.

KEY WORDS — auricular cartilage, costochondral cartilage, laryngotracheal reconstruction, pediatric airway stenosis, pediatric stridor, subglottic stenosis, tracheal stenosis.

INTRODUCTION

Subglottic stenosis (SGS) is a significant cause of morbidity and death in the pediatric population. It is the third most common congenital laryngeal anomaly and occurs in 2.6% to 8.3% of previously intubated neonatal intensive care unit graduates.[1-5] The pediatric otolaryngologist is often called on to aid in the diagnosis and management of this difficult problem. The treatment plan should be individualized for each patient and is based on the patient's age and the severity, location, and type of the stenosis.

Treatment modalities for SGS include both endoscopic and open surgical techniques. Less-severe grades of stenosis are amenable to endoscopic treatment, whereas more-severe grades of stenosis require an open procedure. Laryngotracheal reconstruction (LTR) with a costal cartilage graft (CCG) used to expand the subglottic area has become the most common open surgical procedure since its introduction by Fearon and Cotton.[6] We have previously reported a modification of the CCG-LTR that uses autogenous auricular cartilage grafts (ACGs) instead of CCGs, and we have included these patients in this update.[7] Their inclusion has provided a longer follow-up on the original patients and an increased number of outcomes, resulting in several new recommendations that will be discussed.

MATERIALS AND METHODS

A computer-assisted search of the medical records at St Louis Children's Hospital in St Louis, Mo, was performed. All patients who had an ACG-LTR in the 12-year period January 1985 to December 1996 were identified. Standard demographic data, preoperative diagnosis, cause of the stenosis, operative procedure, complications, and outcome of the reconstruction were recorded. The patients were divided into treatment groups (Table 1[8,9]) depending on which of the following open surgical procedures were initially used to treat their stenoses: ACG-LTR (group 1), anterior cricoid split (ACS; group 2), CCG-LTR (group 3), and tracheoplasty (long- or short-segment stenosis) using either an ACG or a CCG (ACG-TP or CCG-TP; group 4). Group 2 included 1 child in whom an ACS failed and who underwent revision with an Evans-Todd castellated laryngotracheoplasty (Cast-LTP), which has been previously described.[10]

Four surgeons who used essentially the same technique except for suture preference performed the operative procedures. The technique has been described in detail.[7] A reconstructive procedure was deemed a success if a normal, age-appropriate airway was seen

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TABLE 1. TREATMENT OUTCOMES

Group	Patient No. and Procedure Sequence	Stenosis*	Successful Outcome	Overall Success
1 (n = 31); ACG-LTR was first surgery	1-27: ACG-LTR (n = 27)	6 Grade I	6	94%
		17 Grade II	17	
		4 Grade III	3	
	28-31: Multiple LTRs (n = 4)			
	28: ACG ×2	1 Grade II	1	
	29: ACG ×2	1 Grade III	1	
	30: ACG, CCG ×2	1 Grade III	0	
	31: ACG, CCG ×2, ACG	1 Grade II	1	
2 (n = 8); ACS was first surgery	32-37: ACS, ACG-LTR (n = 6)	2 Grade I	2	75%
		2 Grade II	2	
		2 Grade III	2	
	38, 39: ACS, multiple LTRs (n = 2)	0 Grade I	N/A	
	38: ACS, CCG, ACG, CCG ×2	1 Grade II	0	
	39: ACS, Cast-LTP, CCG, CCG (A/P), ACG	1 Grade III	0	
3 (n = 2); CCG-LTR was first surgery	40, 41: CCG, multiple LTRs (n = 2)	0 Grade I	N/A	50%
	40: CCG, ACG, CCG (A/P)	1 Grade II	1	
	41: CCG, CCG, ACG (4 quad split)	1 Grade III	0	
4 (n = 2); Tracheal reconstruction	42, 43: ACG-TR (n = 2)			
	42: ACG	1.9-mm short segment	1	50%
	43: CCG (pericardial patch), ACG	2.7-mm long segment	0	

ACG — auricular cartilage graft, LTR — laryngotracheal reconstruction, CCG — costal cartilage graft, ACS — anterior cricoid split, N/A — not applicable, Cast-LTP — castellated laryngotracheoplasty, A/P — anteroposterior, TR — tracheal reconstruction.

*From 1984 to 1994, system described by Cotton[8] in 1984 was used. From 1995 to 1996, system described by Myer et al[9] in 1994 was used.

at the postoperative endoscopic examination.

RESULTS

DEMOGRAPHICS

Forty-three patients underwent airway reconstruction using ACGs at St Louis Children's Hospital between January 1985 and December 1996. There were 32 boys and 11 girls, for a male-to-female ratio of 2.9 to 1. Their ages at the time of the first LTR ranged from 1 to 168 months, with a median age of 13 months. Seventeen patients had a history of an uncomplicated full-term birth, whereas 26 patients were born prematurely, with a median age of 31 weeks. The following diagnoses were identified: 15 with recurrent croup, 15 with bronchopulmonary dysplasia, 6 with reactive airway disease, and 20 with an indwelling tracheotomy. Associated comorbidities included the following: tracheoesophageal fistula, acromesomelic dysplasia, laryngeal cleft, 8/13 translocation, pulmonary artery sling, Sturge-Weber syndrome, Down syndrome, and VATER association.

CAUSE OF STENOSIS

The records were reviewed to determine the cause of the stenosis in our study group of 43 children. Thirty-two children had a history of endotracheal intubation, and 28 of them had records available for review. The median time of intubation in these 28 patients was 43.5 days. Ten children had no identifiable cause for their stenosis and were thought to have a congenital SGS. Congenital SGS is usually considered to be a more benign condition, which we treat with a wait-and-watch attitude. The indications for surgery in this group of children with congenital SGS included severe recurrent croup requiring hospitalization (n = 7), failure of an attempt at decannulation (n = 3), and the presence of severe reactive airway disease and/or bronchopulmonary dysplasia that complicated their airway management. Five children had a history of both intubation and an elliptical cricoid cartilage and were thought to have a multifactorial cause for their stenosis. One child had a history of laryngeal trauma that was thought to be responsible for his SGS.

PROCEDURES

The 43 patients were divided into 4 groups based on their initial open surgical procedure (Table 1). These 43 patients underwent the 69 open surgical procedures listed in Table 1. A primary procedure is defined as the first open surgical procedure performed on the airway. We consider an ACS (technically a laryngotracheoplasty) a primary open procedure. Any subsequent open surgery is defined as a revision procedure. The Cotton classification and outcomes are reported for each treatment group in Table 1. There were 46 procedures using ACGs, and the complications are listed in Table 2.

Group 1. Group 1 consisted of 31 patients with SGS whose first open procedure was a primary ACG-LTR. Two of these patients also had a posterior cricoid split due to anterior and posterior SGS. Seven of these

TABLE 2. OPERATIVE COMPLICATIONS IN 46 OPERATIONS WITH AURICULAR CARTILAGE GRAFTS

Operative Complication	*Occurrence*
Granulation tissue at graft site*	13
Pulmonary (atelectasis, pneumonia)	12
Postoperative web	2
Tracheomalacia	2
Donor site keloid	2
Cervical emphysema	2
Neuromuscular fatigue	2
Accidental extubation	1
Cervical incision dehiscence	1
Graft loss	1
Sinusitis	1
Cervical abscess	1
Airway keloid	1

*In each case, only very small amounts of granulation tissue were found, except in 1 case, in which it was so great that revision ACG-LTR was necessary.

patients previously underwent an unsuccessful endoscopic laser treatment. The ACG-LTR was successful in 6 of 6 patients with a grade I stenosis, 17 of 19 patients with a grade II stenosis, and 3 of 6 patients with a grade III stenosis. There were 5 initial treatment failures, for an 84% initial (primary ACG-LTR) success rate.

Four of the 5 patients with treatment failure underwent revision surgery. Two patients (Nos. 28 and 29) underwent successful revision with an ACG-LTR. One patient (No. 30) required 2 more revisions with a CCG-LTR, which failed. One patient (No. 31) required 2 revisions with a CCG-LTR followed by a third revision using an ACG-LTR, which was successful. The remaining patient with grade II failure died at home of a surgically unrelated cardiac event 3 months after surgery. His postoperative course was complicated by partial graft loss requiring tracheotomy. The overall success rate for achieving a normal-caliber airway was 94% in group 1.

Thirteen patients had a tracheotomy before their first LTR. Nine patients were successfully extubated or decannulated as a result of their initial procedure, and 2 as a result of a subsequent procedure. There were 2 decannulation failures. One patient was lost to follow-up 6 years after surgery. He was never decannulated, but remained free of stenosis at his last visit 6 years later. The second patient has acromesomelic dysplasia and obstructive sleep apnea. He remains tracheotomy-dependent, although his operative site is free of stenosis. If a successful outcome is defined as decannulation instead of a normal-caliber airway, then the initial success rate would be 77%, for an 87% overall success rate.

The other 2 patients required a postoperative tracheotomy for airway control. One patient's graft failed. He died of a surgically unrelated cardiac event at home, before decannulation, 3 months after surgery. The second patient required 2 more revision surgeries with a CCG and died at home of an unrelated cause with persistent stenosis.

Group 2. Group 2 consisted of 8 patients with SGS whose first open procedure was a failed ACS. They all went on to have revision surgery. There were 6 patients (Nos. 32 through 37) with grade I (n = 2), II (n = 2), and III (n = 2) SGS who then underwent successful revision with a primary ACG-LTR. These 6 patients had an average age of 8.7 months. The remaining 2 patients (Nos. 38 and 39), with grade II and III stenoses, underwent revision with a CCG-LTR and a Cast-LTP, respectively. They each had a total of 4 unsuccessful revision LTRs using both ACGs and CCGs, which failed. In the patients (n = 6) who had an ACG-LTR after a failed ACS, the success rate was 100%. When patients 38 and 39 are included, who underwent revision initially with a CCG-LTR and a Cast-LTR, respectively, after their failed ACS, the overall success rate drops to 75% for group 2.

Of the 6 patients who were successfully treated with a primary ACG-LTR, 3 were tracheotomy-dependent and 3 were still intubated at the time of surgery. Five of these patients were extubated as a result of their single-stage LTR, and 1 patient, who was stented with a No. 7 Aboulker stent, was decannulated 30 days later. The remaining 2 patients who required multiple procedures remained tracheotomy-dependent.

Group 3. Group 3 consisted of 2 patients with grade II and grade III SGS, respectively, whose first open procedure was a CCG-LTR. Each patient had a tracheotomy before the CCG-LTR. Each patient went on to require multiple surgeries with both an ACG and a CCG, with 1 successful outcome (No. 40). Both patients (Nos. 40 and 41) were tracheotomy-dependent, and only 1 was decannulated after his third procedure, a CCG-LTR. This constitutes a 50% overall success rate. Use of auricular cartilage was not successful in this patient group.

Group 4. Two patients underwent an ACG-TP. The first patient (No. 42) was a 2-month-old with a 1.9-mm tracheal airway who underwent successful repair with an initial ACG. The first attempt at extubation on day 7 failed, but he was successfully extubated on day 9. His postoperative course was complicated by pneumonia and neuromuscular fatigue. The second patient (No. 43) was a 4-month-old with a long-segment 2.7-mm tracheal stenosis. He initially underwent repair with a pericardial patch and a CCG. He had a failed extubation on postoperative day 7 and underwent revision with an ACG used to provide support to an area of tracheomalacia. This procedure failed, and the patient required a tracheotomy. He presently requires monthly laser treatments of his persistent severe distal tracheal stenosis.

DISCUSSION

Laryngotracheal expansion surgery is a common modality used to treat children with SGS. Autogenous CCGs are the most popular material used to expand the subglottic area; however, they have potential for creating a pneumothorax. The senior author (R.P.L.) has previously reported on a successful modification of this technique using ACGs.[7] This graft is harvested from the cymba conchae through a postauricular incision and results in minimal donor site deformity and morbidity. In our present series of 46 procedures using ACGs, there were 1 donor site infection and 2 postauricular keloids.

Auricular cartilage grafts have been used for tracheal and subglottic reconstruction since the turn of the century.[11] In 1950, Caputo and Consiglio[12] reported the successful reconstruction of a tracheal stenosis with an ACG. In 1977, Zehm[13] reported on the use of auricular composite grafts (skin and cartilage) in the treatment of "laryngotracheal zone" stenosis. In 1982, Meyer[14] implanted ACGs beneath the buccal mucosa and harvested them as a unit several weeks later to repair the trachea. We have not found composite grafts necessary, because the epithelium will cover 85% of the ACG in 7 days and 100% of the ACG in 14 days, compared with 45% at day 7 and 80% at day 14 for CCGs as reported in an animal LTR model.[15] In 1991, there was a dual institutional report on the success of ACGs in single-stage LTR (SS-LTR).[16] In 1993, our institution reported on the use of ACGs in both staged LTR and SS-LTR.[7] The ACGs were found to be most useful in patients with mild-to-moderate short-segment (<1.5 cm) SGS.

The physical properties of an ACG make it an ideal grafting material for mild-to-moderate SGS. The caliber of the ACG closely matches the strength and thickness of the tracheal cartilage. The graft has a natural arch, which will increase the cross-sectional area of the airway if the concave surface is placed toward the lumen. This is why an ACG has the potential to create a larger lumen than a CCG, which has a flat profile. The arch also helps to prevent prolapse of the graft into the airway. The ACG has been shown to epithelialize more rapidly than the CCG, both in an animal model and in the authors' clinical impression.[15] This decreases the amount of granulation tissue formation during the healing phase.

The treatment of SGS in the neonate or premature infant can be difficult. Although there is no lower weight requirement for open surgical repair of SGS, most patients weigh more than 10 kg at the time of their CCG-LTR.[17] However, the otolaryngologist is frequently consulted on patients with a history of multiple failed extubations who weigh less than 10 kg. Cotton and Seid[18] originally introduced the ACS as an alternative to tracheotomy in this population. Since then, the indications have been expanded to include infants with mild-to-moderate SGS. This operation has a success rate of 71% for primary ACS and 85% for secondary ACS.[19]

Patients with failed ACS treatments are then reintubated and become candidates for a repeat ACS, a single-stage or staged LTR, or a tracheotomy. These patients, or patients who present later in life with SGS, most commonly undergo reconstruction with a CCG. There is, however, a reluctance to perform a CCG-LTR in neonates or premature infants because of the possible morbidity of harvesting a CCG and the technical difficulty of sewing the graft into place. This can delay a patient's definitive reconstruction until sufficient growth has been achieved to allow for harvesting and sewing the CCG safely.

We have harvested auricular cartilage from the cymba conchae using a postauricular incision in premature infants as young as 1 month of age, with only minimal donor site morbidity. This has allowed us to use ACGs for LTRs in neonates and premature infants. The median age of the patients in group 1 (n = 31) was 13 months. Eighty-four percent of these cases were extubated or decannulated. The median age of the patients in group 2 who had an ACG-LTR after failure of an ACS (n = 6) was 7.5 months. All 6 of these patients were extubated or decannulated.

Auricular cartilage proved to be a very useful grafting material for the 6 patients in group 2 who had their ACS revised with an ACG-LTR (100% success) and for all 31 patients in group 1, who had a primary ACG-LTR (84% success). These results are better than those reported for primary ACS and are comparable to those reported for secondary ACS. Unfortunately, we are unable to preoperatively identify which patient will benefit from a primary ACG-LTR instead of an ACS. We have become more liberal about performing a primary ACG-LTR in this population, because of the superior success rates and low morbidity.

We have developed treatment recommendations for the use of primary ACG-LTR based on our treatment results in grade I (100%; n = 6), grade II (89%; n = 19), and grade III (50%; n = 6) stenoses. We recommend primary ACG-LTR for treatment of grade I or II short-segment SGS. We have had limited success with ACGs in grade III stenosis. We feel that a staged reconstruction using CCGs is more appropriate with a grade IV stenosis.

There has been limited experience of using auricular cartilage to revise a failed auricular or costal cartilage LTR in grade II (50%; n = 4) and grade III (33%; n = 3) stenoses. Of the 3 successes, 2 were in patients who had their failed ACG-LTR revised with auricular cartilage. Despite this, we are still hesitant to use auricular cartilage to revise a failed auricular or costal cartilage LTR. However, we do recommend using auricular cartilage to revise failed ACSs. We had a 100% success rate in achieving a normal-caliber airway in our grade I (n = 2), grade II (n = 2), and grade III (n = 3) patients.

In conclusion, auricular cartilage is a useful grafting material for LTR in the pediatric population. It closely resembles the strength and width of the tracheal cartilage and can be used effectively for a grade I or II stenosis. It has the advantage of having minimal donor site morbidity and can be relatively safely harvested in the premature infant. The ACG-LTR is more successful than the ACS operation in treating mild-to-moderate SGS in the premature infant. We have had limited success with ACGs in grade III stenosis and are reluctant to use them in grade IV stenosis, staged reconstruction, long-segment SGS, long-segment tracheal stenosis, or revision of a failed auricular or costal cartilage LTR.

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EFFECT ON FAMILIES AND CAREGIVERS OF CARING FOR A CHILD WITH LARYNGOMALACIA

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We used a retrospective chart review and telephone interviews using a standard format to 1) measure the outcome and psychosocial costs of caring for a child with upper airway obstruction due to laryngomalacia and 2) develop an epidemiologic instrument that measures the impact on the lives of families who have a child with laryngomalacia. Forty-four patients had laryngomalacia documented by endoscopy between 1993 and 1997. Complete follow-up information through 1998 was available on 26 patients. Of the 44 patients, 37 were managed expectantly and 7 (16%) required surgical intervention. Polysomnograms were obtained on 11 before neonatal discharge. Twelve patients wore an apnea monitor for at least 1 month. The quality of life analysis found that the majority of caregivers were not substantially affected by caring for the stridorous child. Loss of sleep and heightened anxiety were commonly reported by these caregivers. Younger parents or parents with fewer than 3 other children were more affected by caring for a child with laryngomalacia. There was no substantial loss of workdays, increase in doctor visits, or difficulty in obtaining day care. Caring for a child with laryngomalacia results in minor disruption of a family's lifestyle.

KEY WORDS — child, endoscopy, infant, laryngeal surgery, laryngomalacia, outcome, parent, stridor.

INTRODUCTION

Laryngomalacia is the most common cause of stridor in infants.[1] The prevalence of laryngomalacia is unknown. During inspiration, structures of the supraglottic larynx collapse toward the laryngeal inlet, creating narrowing of the airway and turbulent airflow.[2] The cause of laryngomalacia remains in dispute. Some authors believe that there is a weakness in the cartilaginous structures of the supraglottis. Others feel that there is a neuromuscular "immaturity" within the supraglottis that resolves over time. The natural history of laryngomalacia has been described.[3] Typically, stridor develops within the first couple of weeks after birth. It can persist for 18 to 24 months and usually resolves spontaneously. Infrequently, laryngomalacia can be severe, resulting in cyanosis, apnea, or failure to thrive. In such situations of respiratory distress, supraglottoplasty has been shown to be effective in reducing the serious manifestations of laryngomalacia.[2,4,5]

Laryngomalacia is diagnosed by laryngoscopy during spontaneous respiration. Unless there are complicating associated findings or significant airway obstruction (apnea, cyanosis, failure to thrive), most otolaryngologists will counsel families about the benign natural history of the disease. Many families are reassured by counseling, though others will continue to seek medical evaluation because of ongoing concerns about their infant's breathing. It is unknown what happens to families and caregivers of children with laryngomalacia; that is, how does this disease affect family relationships and activities? Certain diseases, such as spina bifida, can cause significant family disruption.[6] Quality of life (QOL) surveys have been developed recently for various diseases and their impact on families.[7,8] Using established techniques, we developed a survey for parents and other primary caregivers to assess what effect a child with laryngomalacia has on the lives of these adult caregivers. We also correlated the results of the survey with medical and family data provided by the chart or volunteered by the parents.

METHODS

We retrospectively reviewed the charts of all patients evaluated by laryngoscopy and found to have laryngomalacia at Oregon Health Sciences University (OHSU) from 1993 to 1997. Data were obtained regarding age of onset, age at diagnosis, diagnostic modalities, associated medical conditions, home monitors, use of emergency departments (EDs), and hospitalizations for upper airway obstruction. Those parents who could be reached by phone were then interviewed about the current medical condition of the child and the age of resolution by one of us (H.A.M.). Also, a consent statement was read to each participant by one of us before administering the QOL survey. A separate phone interview was then conducted by the Oregon Survey Research Laboratory (S.M.J.)

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TABLE 1. SYMPTOMS ASSOCIATED WITH LARYNGOMALACIA

Symptom	*No.*	*%*
Severe retractions	16	36
Poor feeding	9	20
Cyanosis	7	16
Apnea	7	16
Failure to thrive	5	11
Regurgitation	3	7

using the QOL instrument to measure the effects that the child's chronic upper airway obstruction may have had on the parents' lives. Professional interviewers were used to conduct the QOL phone survey. These interviewers were trained to reduce the response bias by not leading respondents to give the answer that they think one wants to hear. They were also blinded to any hypothesis or theory about the outcome. Every potential respondent had an equal chance of being contacted (assuming they were in town during the survey field period) because of computer control of telephoning the sample. In addition, appointments were made with many of the respondents, so they were called on the day and time of their choosing. A very high response rate was thus achieved, based on the availability of current telephone numbers. Interviews were conducted in English or Spanish.

The methodology and instrument were reviewed and accepted by the Institutional Review Boards at OHSU and the University of Oregon.

RESULTS

There were 44 children with a diagnosis of laryngomalacia made by 1 of 3 staff otolaryngologists at OHSU. Twenty-two (50%) were girls. The average age at which stridor was first heard by the parents or health care provider was 7 days (median, 1 day; range, 0 to 91 days). All but 1 patient received a diagnosis at the bedside or in the clinic with flexible fiberoptic laryngoscopy. One patient's diagnosis was initially made by direct laryngoscopy. The average age at diagnosis was 77 days (range, 2 to 320 days). Of the 44 patients, 19 (43%) underwent direct laryngoscopy and bronchoscopy. The indications for operative endoscopy varied among otolaryngologists, and no pattern was evident upon review of the various indications. Five of the 19 patients (26.3%) who had bronchoscopy were found to have synchronous airway lesions. None of these were severe enough to warrant independent therapy (eg, laryngotracheoplasty for subglottic stenosis). Other diagnostic procedures done to evaluate the child's upper aerodigestive tract included 9 uses of barium pharyngoesophagrams and 3 uses of pH probes for reflux and/or feeding problems.

TABLE 2. COMORBIDITIES ASSOCIATED WITH LARYNGOMALACIA (N = 44)

Comorbid Condition	*No.*	*%*
GERD	6	13.6
Prematurity	4	9.1
Developmental delay	4	9.1
Reactive airway disease	3	6.8
Cerebral palsy	2	4.5

GERD — gastroesophageal reflux disease. Eight other diagnoses had prevalence of 1 in this cohort: central apnea, agenesis of corpus callosum, Arnold-Chiari II malformation, atrial septal defect, Wolff-Parkinson-White syndrome, chronic lung disease of infancy, aberrant right subclavian artery, and hydronephrosis.

There were several other symptoms associated with laryngomalacia, and these are listed in Table 1. Fourteen patients had comorbid diagnoses that required additional medical therapy. (Some patients had more than 1 comorbidity.) These other problems are listed in Table 2.

Seven patients (15.9%) required surgical intervention to relieve their airway obstruction. There was no single indication that prompted supraglottoplasty, though each patient was described as having "severe" laryngomalacia. One patient had an epiglottic cyst and laryngomalacia (felt to be independent findings by the surgeon). That patient was treated by marsupialization of the cyst and bilateral supraglottoplasty. The other 6 patients underwent supraglottoplasty (4 unilateral, 2 bilateral) alone. One of these patients underwent repeat bilateral supraglottoplasty and ultimately a tracheotomy for persistent upper airway obstruction with cyanosis (measured desaturations) and apnea (captured on memory apnea monitor). The tracheotomy resolved the obstruction, and the patient was decannulated 13 months later without sequelae.

Parents were asked about any monitoring at home (pulse oximetry or apnea monitor) and whether a polysomnogram was obtained during a hospitalization. The frequency of monitoring in our patient population is listed in Table 3. Parents were also asked about the frequency of acute exacerbations, defined as "croup" or ED visits or hospitalizations. There were 28 patients (64%) for whom these data were available. Of these 28 patients, 7 (25%) had a "croup"

TABLE 3. HOME MONITORING AND POLYSOMNOGRAPHY PERFORMED FOR PATIENTS WITH LARYNGOMALACIA

	Yes	*No*	*No Data*
Apnea monitoring	13	23	8
Pulse oximetry	4	30	10
Polysomnography	11	24	9

All patients who had pulse oximetry had concomitant apnea monitoring.

TABLE 4. OUTCOMES SURVEY QUESTIONNAIRE

1. How many months did your child make noise while breathing?
2. How many months old was your child when his/her breathing first alerted you to a problem?
3. How many months old was your child when his/her breathing was at its worst?
4. How many months was your child's breathing affected by laryngomalacia?
5. How many episodes of croup did your child suffer?
6. How many times was your child hospitalized for croup?
7. How many times did your child's croup episodes require emergency room treatment?
8. Is your child over his/her breathing problem now?
9. Does your child have a tendency to easily get sick or develop chronic illnesses?
10. How often did your child's breathing affect his/her feeding?
11. How often did your child's breathing problem affect his/her activities (eg, crawling, walking)?
12. How often did your child's breathing problem affect his/her ability to gain weight?
13. How often did your child's breathing problem affect his/her daily schedule?
14. How often did your child's breathing problem affect your sleep?
15. How often did your child's breathing problem affect your work?
16. How often did your child's breathing problem affect your ability to get together with friends, have people over, or go to social engagements?
17. How often did your child's breathing problem affect your ability to find a baby-sitter?
18. How often did your child's breathing problem affect the other children in your family?
19. How often did your child's breathing problem make you feel anxious, nervous, afraid, or distressed?
20. How often did you and your spouse or partner disagree about your child's breathing?
21. How often did these disagreements lead to serious conflict between you and your spouse?
22. Over time did you become used to the noise your child made while breathing?
23. How many weeks or months did this take (to get used to your child's breathing)?
24. What is the best estimate of the total amount of money you have had to pay because of your child's breathing problem, not counting insurance?
25. Were you the primary caregiver for your child in the time period when he/she had breathing difficulties?
26. Approximately how many hours per week were you responsible for your child's care during the period of time the child had breathing difficulties?

The last questions were directed to obtain demographic information about the caregiver.

27. In what year were you born?
28. How many months and years were you and your spouse or partner together as a couple before the birth of your child who has the breathing problem?
29. How many children do you have altogether (including the one with laryngomalacia)?
30. In order of birth, which child had the breathing problem?
31. Before we end the interview, is there anything else you would like to add about your child's breathing problem that we did not ask you about?

event, 11 (39%) presented to the ED at least once because of airway concerns, and 5 (18%) were admitted to a hospital because of an airway problem. All patients who were hospitalized presented to the ED.

To test whether use of home apnea monitoring plays a role in preventing (or predicting) use of the ED, we used Fisher's exact test to test this hypothesis. There was no significant effect of whether apnea monitoring was used or not in the use of the ED by these patients with acute exacerbation of upper airway obstruction ($\chi^2 = 0.007$, p = 1.0).

From the eligible population of 65 parents and caregivers, 44 caregivers (68%) agreed to participate. These 44 caregivers represent 32 patients from the 44 patients identified with laryngomalacia. There were a total of 30 questions asked of each caregiver. The questions (outcomes survey instrument) are listed in Table 4. The first 8 questions were asked as a measure of test-retest reliability of the participants. There was a very good match between the values recorded during the medical phone survey and those recorded during the administration of the outcomes survey. For example, the median ages reported for the onset of stridor were identical (1 month old), and the numbers of ED visits reported corresponded within 1 visit; those patients who did not seek emergency care were identical between the surveys.

Questions 10 through 21 (Table 4) were presented so that the responder was asked to consider his or her life during those months in which the child with laryngomalacia had the worst problems with his or her breathing. The answers to these 12 questions were

TABLE 5. EFFECT OF PARENTAL AGE ON THEIR QUALITY OF LIFE WITH CHILD WITH LARYNGOMALACIA

Question	*Response*	*Age <30 (N = 15)*	*Age 30-39 (N = 21)*	*Age ≥40 (N = 8)*
Feeding	Rarely or never	33.3	57.1	62.5
Sleep	Rarely or never	26.6	28.6	50.0
Work	Rarely or never	60.0	57.1	62.5
Social	Rarely or never	46.7	61.9	62.5
Babysitter	Often or always	33.3	28.6	0
Anxiety	Often or always	66.7	47.6	37.5
Disagreement	Rarely or never	80.0	85.7	62.5

Data are percentages. Please see Table 4 for complete text for each question. Possible responses for each question are always, often, sometimes, rarely, and never.

always, often, sometimes, rarely, or never. Most caregivers (>50%, confidence interval of ±8.4) indicated that their lives and the child's life were rarely or never affected by laryngomalacia. For example, 50% of the respondents felt that their child's breathing problem rarely or never affected their daily schedule. Likewise, 80% (35 of 44) felt that they rarely or never had disagreements about the child's breathing. Work was never affected in 43% (19 of 44), and rarely affected in 16% (7 of 44). However, 57% (25 of 44) stated that their sleep was often or always affected by the breathing problems of their child. And 84% (37 of 44) sometimes, often, or always felt anxious or afraid about their child's breathing.

On further analysis, correlation of demographic

[redacted]

ents with ≥4 children (including the child with laryngomalacia) were <40 years old. Also, stress within a relationship was worst if the couple had been together less than 4 years (Table 7).

There were 6 responders whose children had supraglottoplasty. (One child and family were lost to follow-up.) Although the survey was not designed to specifically ask about differences on items measured before and after surgical intervention, important differences were found between the group who had supraglottoplasty (SURG) and those patients who were expectantly managed (EXP). These differences are seen in Table 8. Each of the 7 questions demonstrated significant differences in responses to a child with severe laryngomalacia that required supraglottoplasty

TABLE 7. EFFECT OF LENGTH OF TIME ADULT CAREGIVERS WERE TOGETHER BEFORE ARRIVAL OF CHILD WITH LARYNGOMALACIA

Question	*Response*	*≤4 y (N = 14)*	*5-9 y (N = 18)*	*≥10 y (N = 12)*
Feeding	Rarely or never	42.8	38.9	75.0
Sleep	Rarely or never	28.5	27.8	41.7
Work	Rarely or never	57.1	61.1	58.3
Social	Rarely or never	42.9	61.1	66.7
Babysitter	Often or always	28.5	27.8	16.7
Anxiety	Often or always	57.1	66.7	25.0
Disagreement	Rarely or never	64.3	83.3	91.7

Data are percentages. Please see Table 4 for complete text for each question. Possible responses for each question are always, often, sometimes, rarely, and never.

pecially over time. However, only 50% of the parents from the SURG group accommodated, whereas 86.8% (33 of 38) of the parents from the EXP group accommodated.

DISCUSSION

The effects of laryngomalacia on a child are well documented. The stridor develops within the first 2 months of life and is generally a self-limited illness that resolves without intervention.[1] Upper airway obstruction is further evident with retractions and can be positional. Most patients are treated by conservative management with the expectation that the symptoms will resolve by 18 to 24 months of age. However, in 1 study that measured flow-volume loops in older children who had been found as infants to have laryngomalacia, Macfarlane et al[9] found measurable limitations to airflow on inspiration, although this did not pose a clinically significant problem for their patients.

Supraglottoplasty has been used for those patients with severe or life-threatening symptoms of upper airway obstruction associated with laryngomalacia. Frequently cited indications for supraglottoplasty are cyanosis, obstructive apnea, failure to thrive, and dyspnea at rest.[2,5] The efficacy of supraglottoplasty has been demonstrated by clinical resolution of these severe symptoms[2,5] and by physiological measures.[10] Occasionally, some patients do not respond to removal of the redundant supraglottic mucosa, and release of the aryepiglottic folds and tracheotomy becomes necessary.[11]

TABLE 8. EFFECT OF SURGERY (SUPRAGLOTTOPLASTY) ON CAREGIVER'S QUALITY OF LIFE WITH CHILD WITH LARYNGOMALACIA

Question	*Response*	*Surgery (N = 6)*	*Expectant Management (N = 38)*
Feeding	Always	100.0	18.4
Sleep	Often or always	83.3	52.6
Work	Always	66.7	10.5
Social	Rarely or never	16.7	63.1
Babysitter	Often or always	66.7	18.4
Anxiety	Rarely or never	0	18.4
Disagreement	Never	83.3	57.9

Data are percentages. Please see Table 4 for complete text for each question. Possible responses for each question are always, often, sometimes, rarely, and never.

Although much is known about the course of and treatment strategies for a child with laryngomalacia, nothing is published regarding the effect that a child with laryngomalacia has on the parents and caregivers. The survey presented here represents the first attempt to document how the QOL is impacted by laryngomalacia. Development of such an instrument should follow certain principles.[8] There is no other QOL instrument found that tests for a chronic though self-limited illness in infants, but by examining other pediatric QOL instruments and hypothesizing what issues would be relevant to parents, we developed the present survey. In addition, because this was a new area of research, we also asked the open-ended question about whether or not we had overlooked something important. This survey was developed to test the hypothesis that the QOL is not adversely affected in a significant way by caring for a child with laryngomalacia. The methodology for the instrument is likewise well established. In order to prevent our own bias from being expressed by respondents, and in order to guarantee a scientific standard of survey quality, we used the University of Oregon Survey Research Laboratory, which checked the questions for ambiguous wording or unintended bias. The survey was administered by the same organization to guarantee a uniform standard of interviewing by using professional interviewers who were "blind" to our hypothesis and trained to reduce response bias by not leading respondents. One potential problem with this survey, as well as with any in which the patient is a young child, is that it involves proxy measurement.[8] Because this QOL instrument was developed primarily to measure the response that an adult had and how his or her life was affected by laryngomalacia, the issues raised by proxy measurement are not applicable. This QOL instrument appears to be reliable, as there was minimal test-retest variability on the subjects' answers to measurable medical data (eg, admissions to the hospital). This QOL instrument is valid for 2 reasons. First, several otolaryngologists and 2 foster parents (whose responses were not used in the analysis) were administered the survey as part of a "pretest" before testing the 44 parents. Second, the content of each question is a reasonably true and complete statement of the issues that were believed to be relevant to families who had a child with laryngomalacia. This is due to the expertise of the Research Laboratory in developing surveys for a variety of purposes (medical, civic, etc).

In the present study of 44 patients with laryngomalacia, the patient characteristics are similar to those in other reports.[5,12,13] Boys and girls were equally represented, and most patients did not have comorbidities. Most of our patients developed stridor early (average, 7 days; median, 1 day), though they presented for diagnostic evaluation much later (average, 77 days old). A minority of patients had ancillary studies such as rigid endoscopy (43%), polysomnography (31%), and home apnea monitoring (36%). A synchronous airway lesion was found at bronchoscopy in 26% of the patients, and none of these were significant or required additional intervention. This percentage is similar to, though slightly higher than, the findings of others.[4,12] The findings also question the use of home apnea monitoring as a routine adjunct to the care of a child with laryngomalacia, especially if it is being used to prevent costly visits to the ED. There was no significant difference in the use of the ED (or hospitalization) between the group that went home with a monitor and those children who were simply watched by their parents and caregivers. Mothers have reported increased anxiety[14] and fatigue[15] when their infants were sent home with apnea monitors. Because of the small sample and the retrospective nature of the analysis, these observations cannot be generalized.

There were 7 patients (16%) who required supraglottoplasty to relieve the severe manifestations of laryngomalacia. The frequency of surgery for laryngomalacia was consistent with that in other reports from tertiary referral centers.[5] The failure rate for supraglottoplasty (14.3% or 1 in 7) was similar to that of the Froehlich et al[11] report on treatment of severe laryngomalacia.

Results from the survey indicate that most families were able to cope with most of the potential problems that may be faced in caring for a child with laryngomalacia. Daily schedules, work, ability to arrange for a babysitter, and social engagements were rarely or never affected by a child with laryngomalacia. However, it is interesting to note that although most parents did not feel their work was adversely affected, most did feel that their sleep was adversely affected. This apparently conflicting impression can be understood when parent demographics are considered. Most of the parents were under 40 years old, and this group indicated more frequent problems with sleeping. Perhaps these younger parents were able to work more easily in spite of the fatigue due to a lack of sleep. Similarly, those parents with fewer than 4 children were more often unable to sleep well because of their child's laryngomalacia. In may be that with additional parenting experience, stridor at night was not as disturbing for the caregiver. This parenting experience may also explain why there was less reported anxiety toward a stridorous child. Increased anxiety was reported by younger parents and by those who had been in a domestic relationship for less than 10 years. The latter finding may be explained by the additional emotional support a long-term partnership can bring to a parent. Additional psychometric questions would need to be added to the instrument to further explore the basis of these findings.

One concern is that this was a retrospective study with a small sample of parents and patients. Because of the small number of subjects within certain groups (eg, SURG group of 6 parents), individual responses could have a large impact on the results. Also, there were 5 parents surveyed whose impressions of laryngomalacia were 5 to 6 years old (diagnosis in 1993 or 1994), whereas 11 caregivers were surveyed who had memories of their child with laryngomalacia within 1 year of diagnosis (1997). These small numbers, again, prevent meaningful comparisons to evaluate whether time diminished the memory of how severe the effects of laryngomalacia may have been. Nonetheless, the QOL instrument designed to test for changes in parents who have a child with laryngomalacia meets many of the criteria for a clinically useful instrument. As it was first tested in a retrospective manner, the instrument will ultimately need to be tested and validated in a prospective evaluation. Moreover, another aspect of this line of inquiry that remains unknown but could be worth investigating is whether there are important differences between populations of caregivers. That is, are the persuasive abilities of one otolaryngologist compared to another better at convincing a parent that "watchful waiting" is the appropriate therapy for their child with laryngomalacia? A multicenter trial using this instrument would answer this question.

CONCLUSIONS

Laryngomalacia is the most common cause of infantile stridor, and the majority of patients can be managed conservatively with the expectation of resolution without sequelae. An instrument is now available to investigate the QOL changes that may occur when a parent must care for a child with chronic stridor. This QOL instrument is valid and reliable. In this population of 44 parents, most suffered only minor disruptions in their lifestyle. However, parents who may be at greater risk for sleep problems are those with fewer than 3 other children and those under 40 years old. Frequent anxiety may also become a problem for younger parents and those parents in relationships of less than 10 years. A prospective and multicenter study of parents of children with laryngomalacia could clarify the impact of care on the parent's life.

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CHEMODENERVATION OF THE RAT HEMILARYNX WITH PHENOL

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In this study, the injection of phenol into the true vocal fold was evaluated on a rat model as a possible treatment for adductor spasmodic dysphonia. A 10% phenol solution was injected into the right true vocal fold. Quantitative measurement of vocal fold adductory force showed reduction to 35% of the preinjection value 3 months after injection ($p < .05$). Qualitative evaluation by videolaryngoscopy demonstrated maintenance of the normal vocal fold range of motion. Histologic studies showed a transient inflammatory infiltrate and myolysis, while the vocal fold mucosa and the cricoarytenoid joints remained undamaged. Further investigation into the potential use of phenol for treating spasmodic dysphonia is warranted.

KEY WORDS — chemodenervation, hemilarynx, phenol, rat, spasmodic dysphonia.

INTRODUCTION

Spasmodic dysphonia (SD) is a type of focal dystonia involving the muscles of the larynx. Botulinum toxin (Botox) injections to the thyroarytenoid muscles are now routinely used to weaken the laryngeal adductory force, and are considered the treatment of choice for adductor SD.[1-3]

However, Botox therapy has its shortcomings. In a typical treatment cycle, there is suboptimal voice during the initial, breathy phase (7 to 10 days), as well as during the final several weeks before the next injection (as the effects of the Botox gradually wear off). Thus, the "normal voice" phase (2 to 3 months) might be only 50% of any cycle. Botox is also cost-prohibitive for some patients. A longer-acting, less-expensive alternative would be very attractive.

The use of phenol as the injectate might meet these criteria. It has been used successfully for other focal dystonias such as torticollis,[4] hemifacial spasm,[5,6] and writer's cramp.[7] Clinically, it has been injected intrathecally,[8] perineurally,[9] and intramuscularly.[10] Phenol induces axonal degeneration of nerve fibers, with gradual but incomplete recovery of nerve function.[11,12] Intramuscular injections can also cause local myolysis.[13] There is a graded level of injury that is directly related to the concentration of the injected phenol solution.[13,14] The duration of clinical effect can last up to 2 years.[15,16]

The use of phenol in laryngeal dystonia has not been previously reported. One significant concern is whether phenol injected into the thyroarytenoid muscle will damage the surrounding tissues. If the vocal fold mucosa or the cricoarytenoid joint were to be injured, laryngeal phenol injection would be unacceptable. This pilot study addresses the functional and histologic effects of phenol in a rat model.

METHODS AND MATERIALS

Experimental Design. There were 2 phases to this study. The first phase consisted of developing the rat model, including refined laryngoscopic, operative, and injection techniques. In addition, preliminary work was done to determine an appropriate volume and concentration of the phenol for injection into the rat vocal folds. For all experiments, male Sprague-Dawley rats weighing 250 to 300 g were used. A total of 49 rats were used: 20 in phase 1 and 29 in phase 2. The final techniques used in the study are described below.

The second phase consisted of a 10% phenol injection study group (17 rats), a normal saline solution injection control group (5 rats), and a vagal neurectomy positive control group (7 rats). All injections were made into the right true vocal fold; all neurectomies were done on the right side. All procedures were performed by a single investigator. Measurements of true vocal fold adductory force were made at intervals ranging from 2 days to 3 months. Video recordings of vocal fold mobility were made concurrent with the adductory force measurements. Animals were painlessly sacrificed at intervals rang-

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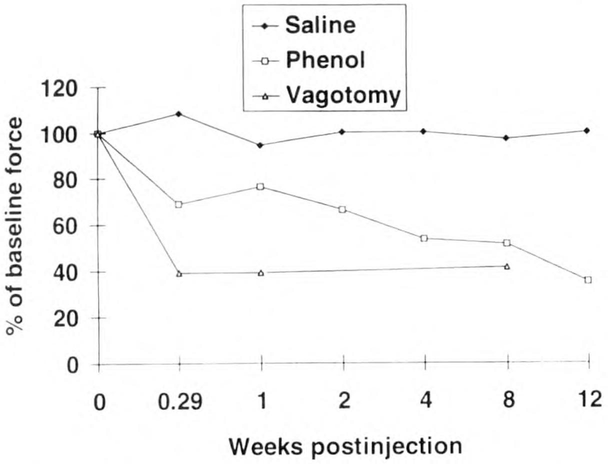

Fig 1. Effects of phenol injection and vagotomy on laryngeal adductory force. Y-axis values are expressed as ratio of measured value to baseline (pretreatment) value.

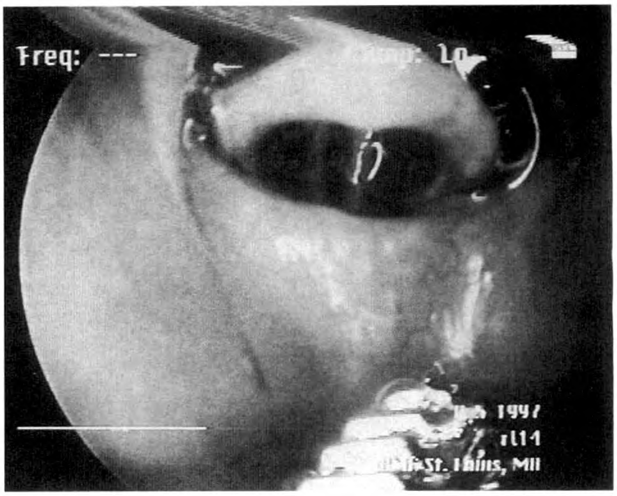

Fig 2. Videolaryngoscopy showing rat larynx immediately after injection. Note fullness of right vocal fold.

ing from 2 days to 3 weeks, and histologic examinations with routine hematoxylin and eosin staining were performed.

Injection Volume and Concentration. To determine the volume of injectate, 8 rat larynges, in vivo and ex vivo, were injected with various volumes of methylene blue ranging from 0.5 to 20 µL. Gross laryngoscopic observations of the spread of the dye were confirmed by subsequent histologic studies. The goal was to keep the spread of the dye within the true vocal fold, with no extension to adjacent structures. The concentration of the phenol was determined from in vivo injections of 3%, 5%, and 10% phenol in normal saline solution into 12 rat larynges. Measurements of the vocal fold adductory force were made at 2 days and 1 week for comparison.

Injection Procedure. Each animal was anesthetized with an intraperitoneal injection of 0.5 mg/kg pentobarbital sodium. Topical lidocaine (4%) was then dripped onto the tongue and soft palate to facilitate laryngoscopy. The rat was placed in the supine position with the mouth held open with stay ties on the upper and lower incisors. Hartmann forceps were inserted to the level of the vallecula and opened; this was accomplished under endoscopic visualization. A 4.0-mm rigid endoscope (Karl Storz), attached to a camera and monitor (Panasonic), was used. This afforded an excellent view of the posterior larynx. Epinephrine 1:1,000 solution was dripped onto the supraglottis and soft palate to reduce edema. A 5-µL Hamilton syringe with a 30-gauge beveled needle was used for the injection. The injections were made transorally into the true vocal fold, just lateral to the anterior aspect of the vocal process. Methylene blue (0.5 µL) was injected in series with the injectate; appearance of the dye submucosally confirmed the correct placement of the injection. After the injection, the rat was intubated with a modified 16-gauge angiocatheter. The rat was then transferred to a warmer and extubated after full recovery from anesthesia.

Adductory Force Measurements. The rat was anesthetized and endoscopic laryngoscopy was performed as described above. The animal was intubated with the 16-gauge angiocatheter. A microtip pressure transducer (Millar, size 2F) was introduced through the angiocatheter into the trachea. The angiocatheter was then removed, leaving in place the transducer. The transducer was slowly withdrawn until the pressure tip diaphragm was between the true vocal folds, as confirmed by pressure readings. Laryngeal closure was induced by dripping normal saline onto the supraglottis and vocal folds. The pressure readings were recorded on a computer with an analog-digital converter and data collection software (Windaq DI-100). Each measurement used was the maximum value that could be obtained repeatedly. After measurements were obtained, the transducer was removed and the rat was intubated and transferred to the warmer to recover from the anesthesia.

Vagal Neurectomy. The rat was placed under anesthesia as described above. The anterior part of the neck was shaved and prepared with Betadine. Lidocaine (1%) was injected locally. A midline, longitudinal skin incision was made, the strap muscles were retracted laterally, and the vagus nerve was located and dissected free. The nerve was bipolar-electrocauterized at the level of the cricoid and divided. The wound was sutured closed.

Histology. Rats were painlessly sacrificed at intervals, and their larynges were harvested and embedded for permanent sections. Sections were done at 50 µm in the coronal plane. The sections were stained with hematoxylin and eosin and examined.

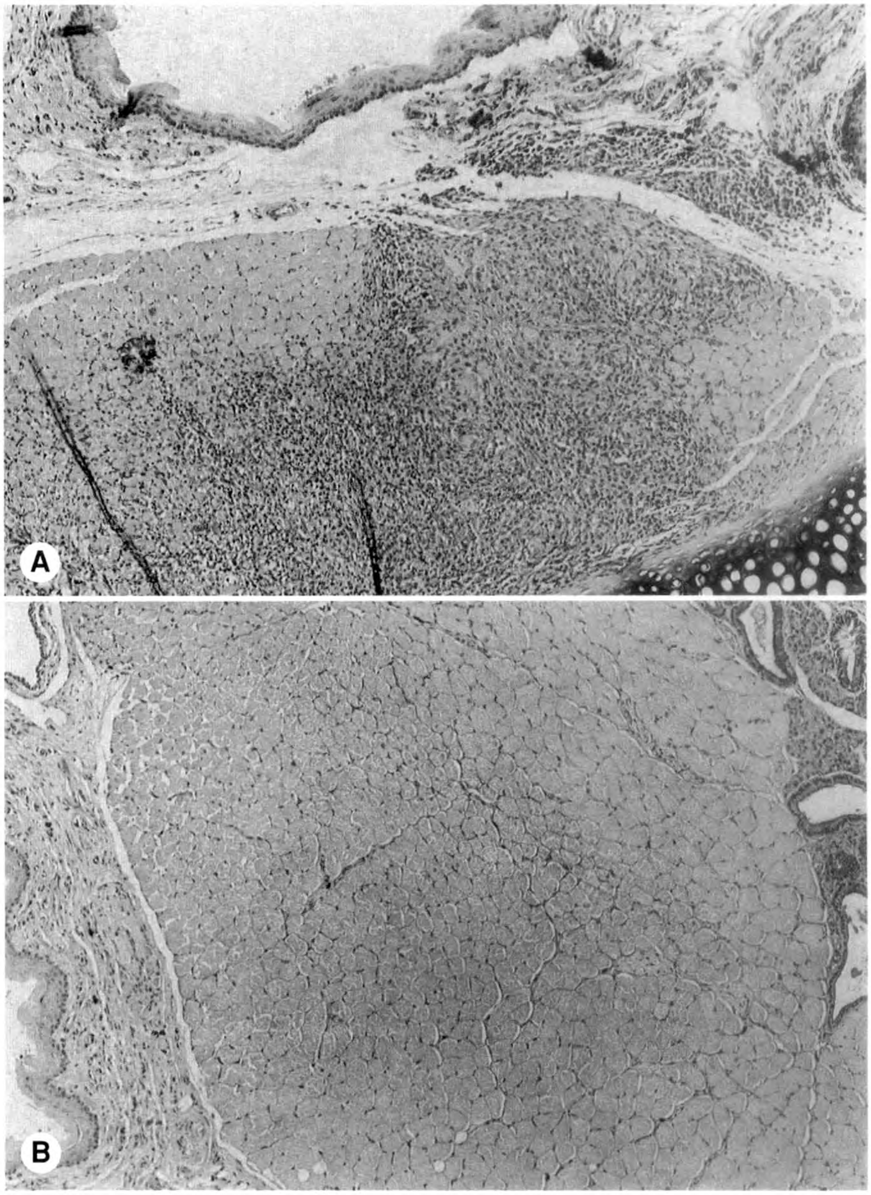

Fig 3. Rat vocal folds following injection with 10% phenol (H & E, original ×250). A) Two days after injection. B) Three weeks after injection.

RESULTS

The refining of techniques for laryngoscopy, injections, and force measurements had a steep learning curve. The initial attempts were characterized by poor visualization, postoperative supraglottic edema, and airway obstruction. The use of the Hartmann forceps, epinephrine, lidocaine, and intubation greatly improved survival. The technique used for adductory force measurement is straightforward. The variability in the measurements was 17%.

A total of 8 rats were used to determine the volume of the injections. Two microliters was the optimum volume. Smaller volumes were judged to have inadequate diffusion, and larger volumes diffused into the subglottis and dissected along the tracheoesophageal wall. Twelve rats were used to determine the concentration of the phenol solution for injections. Concentrations of 3%, 5%, and 10% phenol in normal saline solution were tested. The 3% solution had no significant change in pressure measurement, and the 5% solution had pressure changes lasting only 1 week. The 10% solution provided the desired effect of reduced adductory force lasting more than 1 week.

Seventeen rats underwent 10% phenol solution injections into their right true vocal folds, 5 rats had normal saline injections, and 7 rats had right vagal neurectomies. The adductory force measurements, tabulated as percent change relative to preoperative pressures, are graphed in Fig 1. The normal saline–injected rats showed normal gross vocal fold mobility on videolaryngoscopy, as confirmed by statistically insignificant changes in force measurements. One phenol rat died of unknown causes. Of the 7 rats that underwent vagal neurectomies, 4 were noted to have postoperative stridor. Videolaryngoscopy in the immediate postoperative period showed a paralyzed right vocal fold with consequent reduction in airway patency. Despite intubation during anesthesia recovery, 2 died. Subsequent videolaryngoscopy and force measurements showed a paralyzed right vocal fold in the 5 surviving rats and significantly decreased adductory force. (The remaining measured adductory force was presumed to be coming from the intact, contralateral side.)

Ten rats from the phenol injection groups were followed with interval videolaryngoscopy (Fig 2) and force measurements. Videolaryngoscopy performed immediately after the injections showed decreased mobility of the injected fold and bulging of the mucosa in the area of the injection. Subsequent videolaryngoscopy showed resolution of the mucosal bulging on day 2. The gross decrease in vocal fold mobility resolved at a slower pace, but was no longer evident by the second week. Force measurements showed a decrease in the adductory force to 54% of the preinjection value at 1 month and 35% at 3 months (Fig 1). The decrease was statistically significant beginning 1 month after injection (Student's *t*-test, $p < .05$).

Six rats injected with 10% phenol were painlessly sacrificed at intervals of 2 days to 3 weeks. At 2 days, histologic examination showed a diffuse inflammatory infiltrate both submucosally and intramuscularly (Fig 3A). Large areas of myolysis were visible within the thyroarytenoid muscle; the mucosa remained intact, and the cricoarytenoid joint was undamaged. This picture remained unchanged in the 1-week postinjection specimens. At 2 and 3 weeks after injection, the inflammatory infiltrate was much reduced; there was still evidence of myolysis, but it was less extensive (Fig 3B). The infiltrate seemed more predominant around the neurovascular bundles. No evidence of fibrosis was seen. The mucosa and cricoarytenoid joints remained undamaged.

DISCUSSION

Spasmodic dysphonia can significantly alter a patient's communicative abilities. Botulinum toxin is an accepted form of treatment, but has its disadvantages, including a relatively short duration of action and high cost. Phenol is an inexpensive alternative with a potentially longer duration of action. It has been used successfully in the treatment of other disorders. However, given the delicate nature of the larynx, the effects of phenol on laryngeal tissues and function must be addressed.

The physiological effects of injected phenol have been addressed by many authors.[11,14-17] The adductory force measurements in this study confirm the reduction in muscle function observed in other studies.[11,14] The return of gross vocal fold mobility to normal, while the adductory force remains reduced, agrees with observations made by Bodine-Fowler et al[11] in rat extremity muscles. This fact is encouraging, because this is the desired treatment effect in human patients. The transient decrease in mobility likely represents a volume effect from the injection. The duration of action is still undetermined, but these data suggest that it is at least 3 months.

The histologic tissue effects of phenol have also been studied.[12-14] The mechanism is one of protein degradation, the extent and duration of which are dose-dependent. Our study used a 10% phenol solution, a higher concentration than those in other studies. Nevertheless, the histologic examinations showed no mucosal or cricoarytenoid joint injuries. The observed inflammation and myolysis were shown to improve with time.

The results from this study are encouraging. Further studies are needed to evaluate both the dosage of the phenol and its effect on the larynx. Issues that cannot be addressed with a rat model are the more subtle vocal fold functions, such as mucosal wave production and voice quality; a dog model would allow for such observations. Another possibility that awaits further investigation is the concurrent injection of hyaluronidase with phenol; this may cause greater dispersion of the phenol, allowing a reduction in the phenol concentration and perhaps the extent of tissue injury.[18,19]

CONCLUSION

Intramuscular phenol injections successfully reduced laryngeal adductory force in this rat model for over 3 months, without damaging the vocal fold mucosa or cricoarytenoid joint. Further studies in a canine model are indicated to determine whether phenol might be a good alternative to botulinum toxin for patients with spasmodic dysphonia.

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ARYTENOID ADDUCTION: CONTROLLING VERTICAL POSITION

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In flaccid laryngeal paralysis, the vocal process (VP) is displaced laterally and superiorly. The arytenoid adduction procedure (AA) moves the VP medially and caudally, closing the glottic gap. However, clinical evidence suggests that the VP is more caudal after AA than in physiological phonation. The neurally intact arytenoid is supported by tonic and phonatory activity of the posterior cricoarytenoid muscle (PCA). We hypothesize that a posterior anchoring suture could replace PCA support, achieving a more natural VP location. Cadaver larynges were scanned with computed tomography at rest and after AA, alone or in combination with a second arytenoid suture anchored to either the posterior midline cricoid (PC) or the inferior thyroid cornu (IC). Each posterior suture reduced caudal displacement of the VP during AA, but the glottic gap was wider with the PC suture. In 3 patients undergoing AA for laryngeal paralysis, the IC suture improved arytenoid posture and voice quality.

KEY WORDS — arytenoid adduction, laryngeal paralysis.

INTRODUCTION

The symptoms of laryngeal paralysis vary greatly. Laryngeal paralysis is sometimes detected during routine examination of a patient with no vocal complaints. More often, a patient with laryngeal paralysis has a weak or breathy voice, or even aspiration during swallowing. The severity of symptoms has long been attributed to the "position" of the paralyzed vocal fold.[1] When the paralyzed fold is located near the midline, compensatory activity of the normal side of the larynx can often close the glottis during phonation. Aspiration is rare, and hoarseness responds well to surgical medialization of the membranous vocal fold. However, when the vocal fold is in a more abducted position, glottic incompetence is more severe, and medialization procedures such as vocal fold injection or Isshiki type I thyroplasty are less successful in restoring vocal function.[2,3]

Although several theories have been proposed to explain the variations in symptoms and vocal fold positions in patients with laryngeal paralysis, it is now generally accepted that the relatively abducted position occurs in patients with flaccid paralysis, and that a medial position indicates incomplete paralysis, with residual or regenerated innervation of laryngeal muscles.[4,5] Further, flaccid paralysis results in a complex alteration in glottic configuration and arytenoid posture. The arytenoid of the flaccid vocal fold is rotated so that its vocal process (VP) is displaced rostrally and laterally.[6] Because of the angle between the membranous and cartilaginous portions of the vocal fold, the patient cannot approximate the VPs, even with vigorous hyperadduction of the normal vocal fold. This results in incomplete glottic closure, with a configuration generally referred to as a "posterior gap." Actually, this gap is not truly posterior, but is maximal at the junction of the membranous and cartilaginous portions of the vocal fold, and extends throughout the length of the glottis. The arytenoid adduction procedure (AA) was developed for use in patients with a "posterior gap."[7] The intent is to rotate the arytenoid into a more normal position, by mimicking the action of the lateral cricoarytenoid muscle. The membranous vocal fold is dragged medially by this procedure.

A paralyzed vocal fold also appears shorter than the mobile fold.[6,8] Cadaver studies suggest that the paralyzed vocal fold is not really shorter, but only appears so because of visual foreshortening; the paralyzed vocal fold does not lie in the same plane as the nonparalyzed fold.[9] Successful AA increases the apparent length of the paralyzed vocal fold, probably by rotating it toward the visual plane. However, the fact that AA rarely restores normal appearance suggests that it does not achieve a physiological position of the VP, but rather, one that is too high or too low.[10]

Another characteristic of laryngeal paralysis is anterior "tipping" of the arytenoid, which may even obscure the view of the vocal fold. This is most severe in patients with complete denervation. The arytenoid posture is more normal and erect in patients with

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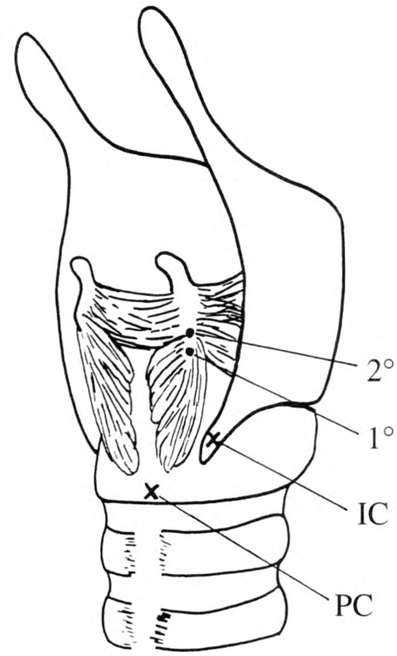

Fig 1. Posterior view of larynx demonstrating attachment of primary arytenoid adduction suture (1°) and posterior anchoring suture (2°) to arytenoid, and loops for posterior attachment to inferior cornu (IC) and posterior cricoid (PC).

incomplete paralysis, as demonstrated by electromyographic activity in laryngeal muscles.[11] The anterior tilting probably results from loss of support by the posterior cricoarytenoid muscle (PCA). The PCA has long been known to contract during phonation.[12] Possible effects of anterior tipping are inferior displacement of the VP and decreased tension in the vocal fold.

Arytenoid adduction does not address arytenoid "tipping." Cadaver studies indicate that AA results in rotation of the arytenoid about an oblique, nearly vertical axis, and does not "rock" the arytenoid posteriorly.[13] Contraction of the PCA results in rotation about an axis roughly perpendicular to that of the AA, with significant posterior "rocking" of the arytenoid.[14] Neither maneuver is associated with significant lateral translation ("gliding"). Thus, it is possible that addition of a posterior suture, to simulate PCA support, could achieve a more physiological position of the arytenoid and thus improve the results of the AA. We compared the effects of 2 different posterior anchoring sutures in excised cadaver larynges and tested the modified AA in 3 patients.

METHODS

Particles (1 × 1 mm) of radiopaque polyethylene tubing were sutured onto 3 freshly harvested adult cadaver larynges, to create radiopaque markers at the anterior commissure, 4 other points on the thyroid cartilage, at the VPs, at the muscular processes and apices of the arytenoid cartilages, and at 5 points on the cricoid cartilage, including the posterior-superior midline. Sutures were placed on the muscular process of each arytenoid, leaving 2 long tails for performing AA. The ends of these sutures were passed loosely through the anterior-inferior thyroid cartilage, but not tightened. A second suture was placed just medial to the muscular process, again leaving long tails. Small suture loops were attached to the inferior cornua of the thyroid cartilage, and to the posterior-inferior cricoid cartilage, to serve as points of attachment for the posterior anchoring sutures (Fig 1).

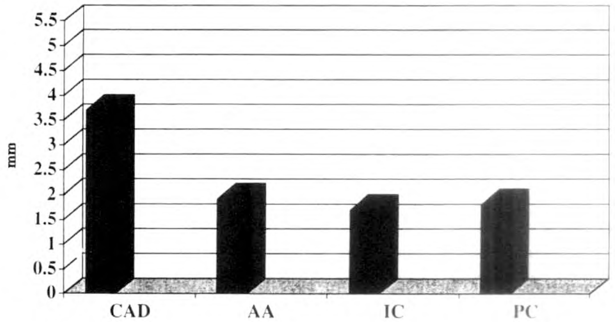

Fig 2. Graphic representation of mean glottic gap (distance between vocal process markers) in cadaveric position (CAD), after arytenoid adduction (AA), and after addition of posterior suture anchored to ipsilateral inferior cornu of thyroid cartilage (IC) or to posterior-inferior cricoid cartilage (PC).

Each specimen was suspended in a saline-filled Plexiglas box. Computed tomography (CT) was performed with the larynx in the cadaveric position, and after bilateral AAs passing the sutures from the muscular process through the inferior edge of the anterior thyroid cartilage. Scanning was repeated twice more: once with the second suture secured to the posterior midline of the cricoid cartilage (PC), and once with the anchoring suture secured to the ipsilateral inferior cornu of the thyroid cartilage (IC). The cricoarytenoid ligaments and cricoarytenoid joints were not disrupted.

Data from the CT scans were analyzed with 3-dimensional imaging software. Multiple CT images were displayed. The images were zoomed, and the contrast was adjusted to help identify the radiopaque markers. Each marker was labeled by defining a fiducial mark at its center. The axial plane was defined as the x-y plane, with x increasing toward the right, and y increasing from anterior to posterior. The z axis was perpendicular to the axial plane, with increasing values from rostral to caudal. The z coordinate of each fiducial mark was determined by its slice position, and the x-y coordinates were determined by the cursor position within the slice, all relative to an arbitrarily chosen origin.

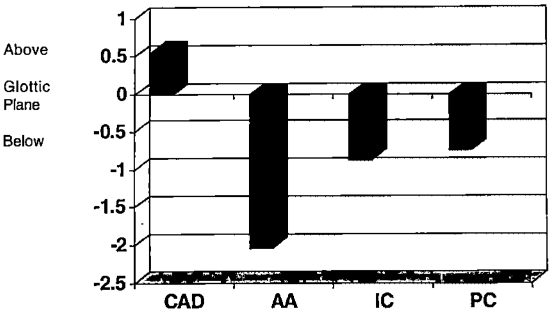

Fig 3. Vertical displacement (mm) of vocal processes from cadaveric position (CAD) by arytenoid adduction, alone (AA) or in combination with inferior cornu (IC) or posterior cricoid (PC) anchoring suture.

Medialization of the VPs was determined by measuring the distance between markers on the VPs. The length of each membranous vocal fold was defined as the distance between the anterior commissure and the corresponding VP. Changes in the rostrocaudal position of each VP were determined relative to a reference glottic plane passing through the anterior commissure and posterior glottic midline and perpendicular to the sagittal plane.

RESULTS

The mean glottal gap was 3.5 mm in the cadaveric position and was diminished by all variations of the AA (Fig 2). There were no significant differences between mean glottal gaps after the standard AA or the modified AA anchoring the suspension stitch to either the PC or the IC.

The rostrocaudal location of each VP was determined in relation to an arbitrary reference plane passing through the anterior commissure and the posterior-superior cricoid cartilage. Figure 3 depicts displacement of the VP from its location in the cadaveric position to that in each of the treatment positions. The AA resulted in the largest inferior displacement. The PC and IC produced smaller magnitudes of displacement, so that the VP was higher than in the AA.

Vocal fold length was not significantly altered by any of the treatment conditions (Fig 4).

DISCUSSION

The cadaver studies indicate that a posterior anchoring suture can alter the position of the VP in the AA. Addition of either suture consistently resulted in elevation of the VP. The effects of medialization of the VP varied somewhat among specimens. Whereas the IC stitch did not significantly affect the medialization achieved by the AA, the PC suture did exert an abductor force in 1 specimen. Additionally, the IC is technically much easier to anchor, particularly in vivo. Hence, of the 2 posterior anchoring sites tested, the IC site appears to be the most promising.

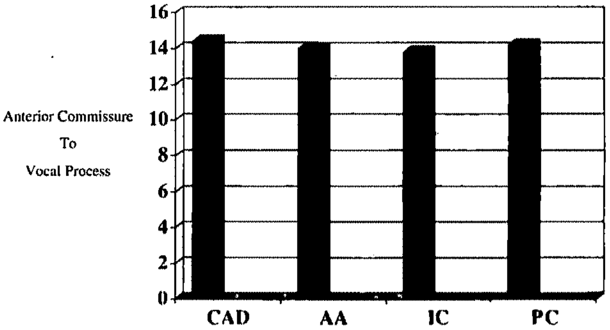

Fig 4. Length (mm) of membranous portion of vocal fold (distance between markers at anterior commissure and vocal process) for cadaveric position (CAD) and after arytenoid adduction, alone (AA) or in combination with posterior suture anchored to inferior cornu (IC) or posterior cricoid (PC).

Differences in the effects of the 2 sutures would be consistent with the recent observation that subtle differences in the vector of traction can significantly affect arytenoid motion.[14,15] Specifically, the compartments within the PCA have significantly different actions. Regional stimulation of the PCA in dogs has demonstrated that the horizontally oriented compartment exerts primarily a "swiveling" action, with rotation primarily about a vertical axis.[15] The vertical and oblique compartments were more effective in abducting the vocal fold and in rocking the entire arytenoid posteriorly. The human PCA contains only 2 compartments, but their actions are similar to those in the canine PCA. The muscle fibers in the human PCA medial compartment follow a more horizontal course than those in the lateral compartment. Studies of simulated contraction of individual compartments indicate that the vertical compartment is more effective in rocking the arytenoid posteriorly, and abducts the VP more widely. The horizontal component produces primarily "swiveling" about a vertical axis, and less actual abduction of the vocal fold.

The PC suture used in this study follows a course midway between those of the vertical and horizontal components, connecting the muscular process and the posterior-inferior cricoid. The PC is effective in elevating the VP, but it also abducts the vocal fold. The IC suture is angled more laterally than either muscle compartment. Thus, it would be expected to provide posterior stability and VP elevation, but less vocal fold abduction.

Preliminary experience in patients supports the potential benefit of the IC anchoring suture. The procedure was performed in 3 patients with flaccid paralysis and no electromyographic evidence of muscle activity on the involved side. In each patient, the arytenoid tipped anteriorly, partially obscuring the

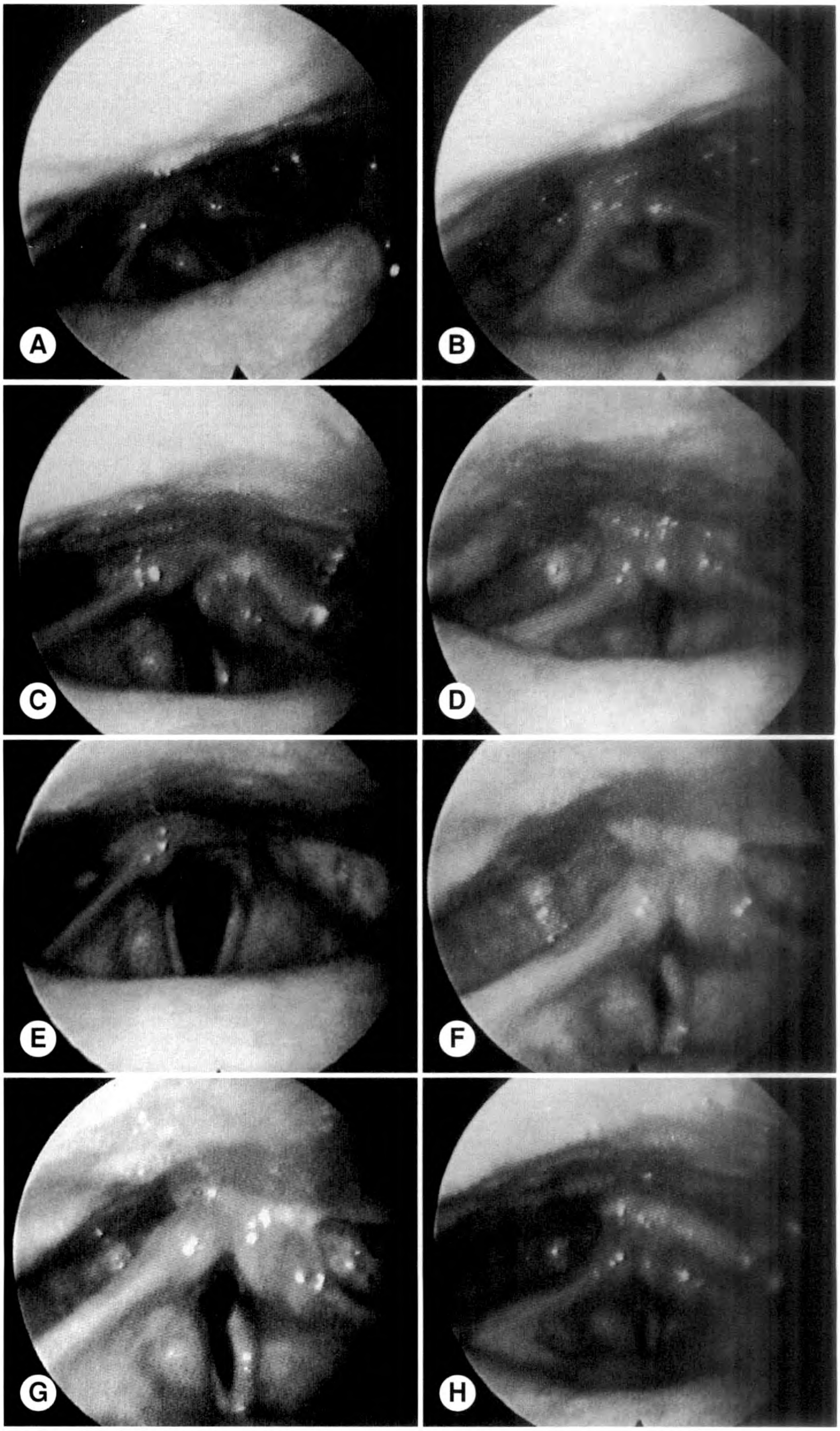

Fig 5. Intraoperative effects of anchoring sutures. Endoscopic photos during A) inspiration and B) phonation before placement of traction sutures. Effects of C,D) adduction suture only, E,F) posterior suture only, and G,H) combined anchoring sutures.

membranous vocal fold, and the vocal fold was paralyzed in a lateral position. A standard AA was performed, and a second suture was placed on the arytenoid, just medial to the primary suture. With the AA suture approximated, the patient was asked to make a sustained phonation, and traction was applied to the anchoring suture. This resulted in a clearer voice, and endoscopy demonstrated a more erect posture of the arytenoid (Fig 5). In this study, we took care to ensure that the anchoring stitch was placed more medially on the arytenoid than the primary AA suture, as it was felt that a stitch originating from the

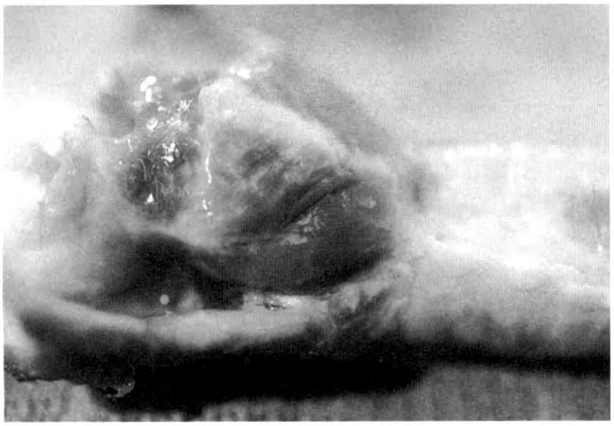

Fig 6. Posterior oblique view of cadaver larynx demonstrating linear medial continuation of muscular process.

same locus or lateral to the primary suture would be more likely to result in cancellation of forces. The muscular process is not just a point, but a linear structure continuous with a small ridge extending toward the VP (Fig 6). The anchoring suture was placed through this ridge, at least 3 mm lateral to the primary stitch.

It is important to note that the AA changes the position of the VP and passively relocates the membranous fold. However, it does not address glottic incompetence due to vocalis muscle atrophy. The AA often must be combined with type I thyroplasty or vocal fold injection.

An alternative surgical procedure for correcting the vertical position of the VP has been described by Zeitels et al.[16] This is termed "adduction arytenoidopexy" and consists of transecting the PCA and using a single suture to achieve 2-point stabilization of the cricoarytenoid joint.[16]

Cadaver studies provide useful information, but do not definitively replicate processes in patients. In living subjects, larynges are frequently not totally denervated, and they are also subject to forces exerted by the contralateral laryngeal muscles and extrinsic laryngeal muscles. Moreover, long-standing paralysis can lead to muscle contracture, with confounding forces. Therefore, caution should be used in extrapolating from cadaver studies to clinical applications. Nonetheless, preliminary observations of the effects of the posterior suspension suture in patients who have undergone the AA are promising. Further clinical study and assessment of vocal outcomes are warranted.

CONCLUSIONS

Flaccid laryngeal paralysis results not only in an abducted vocal fold, but also anterior "tipping" of the arytenoid, with inferior displacement of the VP. Arytenoid adduction as described by Isshiki et al[7] is effective in adducting the VP, but does not correct the anterior tilt and may further depress the VP. These cadaver studies indicated that the addition of a posterior suspension suture in the AA results in less caudal displacement of the VP and a more favorable arytenoid posture. Further clinical study is warranted.

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LARYNGEAL SYNKINESIS REVISITED

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First described in 1982, laryngeal synkinesis continues to play an important diagnostic and therapeutic role following recurrent laryngeal nerve (RLN) injury. Vocal fold motion impairment (formerly called "vocal cord paralysis"), hyperadducted and hyperabducted vocal folds, and certain laryngeal spasmodic and tremor disorders are often best explained by synkinesis. A closer look at these mechanisms confirms that following RLN injury, immobile vocal folds may be nearly normally functional (favorable), or spastic, hyperadducted, or hyperabducted (unfavorable). This has resulted in a *functional* classification of laryngeal synkinesis as follows: type I laryngeal synkinesis, with satisfactory voice and airway (vocal fold poorly mobile, or immobile); type II synkinesis, with spasmodic vocal folds and an unsatisfactory voice and/or airway; type III synkinesis, with hyperadducted vocal folds and airway compromise; and type IV synkinesis, with hyperabducted vocal folds, poor voice, and possible aspiration. This classification facilitates the understanding of laryngeal pathophysiology following RLN injuries and promotes a more scientific basis for management.

KEY WORDS — laryngeal paralysis, laryngeal synkinesis, recurrent laryngeal nerve, vocal fold motion impairment.

INTRODUCTION

"Laryngeal synkinesis" is a state of abnormal laryngeal innervation that may exist following injury to the recurrent laryngeal nerve (RLN). First mentioned in the literature in 1982 and further elucidated in 1989, it is increasingly recognized as a mechanism that explains various laryngeal phenomena following RLN injury. It has not previously been clearly defined, physiologically delineated, or adequately understood. Yet, synkinesis is frequently the state of abnormal innervation, resulting from nerve regeneration and attempted physiological reinnervation, that is seen in larynges with vocal cord "paralysis," "immobility," and "motion impairment," as well as certain spasmodic and dystonic disorders.

In this paper, the author intends to define laryngeal synkinesis, further enumerate the various possibilities for reinnervation resulting from synkinesis, and clarify certain common clinical patterns of synkinetic reinnervation seen by mirror, fiberoptic, or electromyography (EMG) examination.

DEFINITION OF LARYNGEAL SYNKINESIS

Laryngeal synkinesis is a vocal fold motion disorder (intrinsic laryngeal muscular dyssynergy, asynchrony, and/or contractile competition) resulting from misdirected and random regeneration of RLN motor axons (intrinsic) or of surgically implanted nerves (extrinsic) or both (combined). It may be *favorable*, with few or no phonatory or airway symptoms, or *unfavorable*, causing dysphonia, airway impairment, and/or aspiration.

PHYSIOLOGY AND PATHOPHYSIOLOGY

In health, the RLN is capable of remarkably complex neural control of the various neuromuscular functions of the larynx. It contains motor, sensory, proprioceptive, secretomotor (parasympathetic), and, possibly, sympathetic nerve axons. During phonation and coughing, the RLN transmits neural impulses from the nucleus ambiguus to induce synchronous and appropriate vocal fold adduction via innervation to the thyroarytenoid muscle (TA), lateral cricoarytenoid muscle (LCA), and interarytenoid muscle (IA). During these adductive maneuvers, the RLN may stimulate certain portions of the posterior cricoarytenoid muscle (PCA) to contract, although more frequently, major portions of the PCA are inhibited during such adductory vocal fold movements.

Similarly, but conversely, during inspiration, the RLN produces arytenoid and vocal fold abduction (glottal enlargement) by evoking muscular contraction in the most abductive portions of the PCA, while inhibiting the strongest adductor, the LCA. Some, but not all, of this respiratory cyclical stimulation and inhibition is reflexly controlled and triggered by laryngeal, tracheal, and pulmonary stretch and pressure receptors. Accordingly, the function of the normal RLN is quite complex, requiring several nerve and muscle groups to function correctly and appropriately in synchrony throughout the respiratory cycle.

It is not surprising, then, that once an RLN is cut, stretched, cauterized, or otherwise injured, normal laryngeal neuromuscular function is rarely, if ever,

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observed. The situation is not unlike that of the facial nerve, which, if injured, never regains normal function.[1] As a complex nerve containing sensory and proprioceptive fibers in addition to motor axons, the RLN is presumed to undergo regenerative chaos following a transection injury. Sensory fibers may or may not regenerate back to sensory structures, and similarly little is known about proprioceptive fibers' ability to regenerate. It appears that sympathetic and/or parasympathetic axons (in the RLN or adjacent nerves) may have the capability to regenerate to, and complete effective "neuromuscular" connections to, laryngeal muscle.[2,3]

However, it is the motor axons of the RLN that would appear to be most responsible for the various phenomena of laryngeal synkinesis. Most authorities believe that there is very little guidance provided to help regenerating motor axons find their prior appropriate and correct laryngeal muscle, and that they regenerate in random fashion through the distal stump of the RLN even if a segment has been removed. (This is, in fact, the most plausible explanation of recurrent spasmodic dysphonia following RLN resection.[4]) Some axons appear to be able to regenerate directly toward the laryngeal muscles without the structure, aid, and guidance of RLN endoneurial tubules. Others, possibly the most prevalent ones, regenerate via the endoneurial tubules of the distal stump toward the denervated larynx. In proximal injuries, such as intrathoracic lesions, the regenerating axons have several opportunities to exit the "parent" RLN, including through the segmental tracheal sensory nerves (improbable as this is) and the sensory and/or proprioceptive anastomosis of Galen, immediately proximal to the exit point of the abductor branch to the PCA.

Hence, the regenerating motor axons, whether they be formerly phonatory-adductive to the LCA or IA, or formerly inspiratory to the PCA, will most presumably follow the path of least resistance, whether this resistance be pharmacological (as with "axon sprouting inhibitor factor"), mechanical (scar tissue), or otherwise. The PCA's abductor branch exits the RLN near the cricothyroid joint, just under the "cover" of the overlying lower lateral aspect of the thyroid cartilage. This abductor branch, no longer than 3 to 4 mm in length, is the first motor branch to leave the RLN, and hence represents the first exit possibility for regenerating axons to leave the RLN and to establish motor connections. Hence, the PCA appears to have at least 1 advantage over the other 3 muscles in reestablishing neuromuscular connections (functioning motor end plates): its proximal position relative to the regenerating axons and the other 3 muscles (LCA, IA, and TA; see description of type IV synkinesis, below).

Because the PCA is generally known to be characterized by larger motor unit sizes (each normal PCA motor axon innervates 50 to 200 muscle fibers,[5] as compared to the 1 to 20 fibers[6] of the TA and LCA), it is possible that as these regenerating axons pass into the PCA's intramuscular neural network, they may readily sprout and form functioning PCA motor end plates. The neuromuscular effect of this is poorly understand, as it is not known whether an axon with a smaller motor unit, such as a normal TA nerve fiber, is capable of expanding its motor unit size significantly, after nerve regeneration and muscle reinnervation.

What has becoming increasingly clear is that after RLN injury, most larynges that demonstrate vocal fold abnormalities, including "vocal cord paralysis" (actually motion impairment), have innervated laryngeal muscles, as may be confirmed by EMG.[7,8] Some authors believe that even when the RLN is not capable of regenerating axons back to the paralyzed larynx (such as following RLN resection for thyroid cancer, or intrathoracic RLN transection), several adjacent nerves may sprout toward, and reinnervate, the paralyzed intrinsic muscles. These adjacent nerves include terminal fibers of vagal branches to the pharyngeal constrictor muscles, the internal branch of the superior laryngeal nerve, cervical sympathetic and parasympathetic nerves, and poorly understood intralaryngeal branches.[9] Some authors, in fact, believe that true EMG-proven *paralysis* of the laryngeal muscles is rare following RLN injury.

Further, it seems far too simplistic to explain laryngeal synkinesis solely on the basis of regenerative phenomena of the *motor* components of the RLN. Parasympathetic and sympathetic axon sprouting could somehow contribute to motor end plate innervation in laryngeal muscles.[2,3] However, until more is known about adjacent nerve-sprouting mechanisms, and proprioceptive and sensory nerve sprouting, it seems useful to explain synkinesis on the basis of the motor fibers of the RLN and their regenerative capabilities.

It has been shown that more motor axons are destined for innervation of the LCA and TA muscles than for their antagonist abductor muscle, the PCA.[5,6] Fiber typing experiments have elucidated the various myosin heavy chain isoform makeups of the various muscles.[10] Clearly, the TA is a "tuneable" muscle in which motor unit sizes may approximate 1:1 (1 motor axon per 1 muscle fiber), so that certain components of the TA can contract quickly and individually.[6] The LCA, phonatory and adductory like the TA,

SYNKINETIC EFFECTS OF INDIVIDUAL MUSCLES

Muscle	*Normal Function*	*Source of Synkinetic Reinnervation*	*Abnormal Vocal Fold Behaviors*
Thyroarytenoid (TA)	Phonatory, tensing, tuning	PCA axons (LCA axons)	Twitching, spasms, inspiratory medial bulging
Lateral cricoarytenoid (LCA)	Phonatory (and cough) adduction	PCA	Adduction during inspiration
Posterior cricoarytenoid (PCA)	Inspiratory abduction	LCA, IA, TA	Spasms, inappropriate abduction, phonatory air escape
Interarytenoid (IA)	Posterior glottic closure	PCA	Adduction during inspiration

also appears to have a larger proportion of fast-twitch muscle fibers than does the slower-contracting abductor, the PCA muscle. Poorly understood is the effect of reinnervation of former type I muscles by type II nerve fibers, and vice versa. However, it is clear from both animal and clinical EMG studies that when the PCA, LCA, and TA are each innervated both by axons firing during phonation-adduction and by axons firing during inspiration-abduction, little or no effective vocal fold movement can occur.[11] However, certain patients with this synkinetic vocal fold motion impairment may have good-to-excellent voices.[7,12]

SPECIFIC INTRINSIC MUSCLE PERSPECTIVES

The TA appears to be the most important phonatory tuning element of the larynx (see Table). In normal phonation, it thins, tenses, or flattens the vocal fold. If the TA is either hyperinnervated or aberrantly innervated (excessive reinnervation by inspiratory-firing axons), it may demonstrate vocal fold twitching, spasms, or other involuntary movements. It may bulge toward the midline, encroaching upon the inspiratory airway, if there is significant reinnervation by former PCA axons.

The LCA is the most effective adductor muscle for the vocal process and the vocal fold. Accordingly, if the LCA has excessive or significantly aberrant reinnervation, the vocal fold may adduct during inspiration, causing glottic airway compromise.

The PCA's primary function appears to be vocal fold and arytenoid abduction during inspiration. Additionally, it stabilizes the arytenoid and the vocal process during phonation, especially high-pitched phonation. If inappropriately innervated by aberrant regeneration, the PCA may attempt to abduct the vocal fold during phonation, swallowing, or coughing. Other portions of the aberrantly reinnervated PCA may induce abnormal arytenoid movements, which may lower or raise the inferior-superior position of the vocal process (thereby placing the ipsilateral vocal process and vocal fold at a lower or higher level than the contralateral normal vocal process). In addition, aberrant reinnervation of PCA fibers may induce vocal fold and/or arytenoid spasms, twitching, or other involuntary glottic movements.

Possibly the least understood of the 4 intrinsic muscles, the IA appears to assist in arytenoid adductive movements and possibly in controlling the size of the posterior glottic chink. In addition, it may assist the PCA in certain abductive and adductive maneuvers. Hyperinnervation of the IA muscle would be expected to hyperadduct the arytenoid, possibly compromising the posterior glottic airway. Aberrant synkinetic reinnervation of IA fibers by inspiratory PCA axons would be expected to cause the IA to adduct the arytenoid during inspiration, negating abductive efforts by the PCA.

CLASSIFICATION OF LARYNGEAL SYNKINESIS

It is with this physiological background that a classification of synkinesis was developed. In following a large number of patients with "vocal cord paralysis" over many years, particularly with the progressively improving resolution of fiberoptic and rigid endoscopes, I have found it to be apparent that not all "paralyzed" larynges (following RLN injury) are the same. Hence, it is not accurate to say that a patient who has a known or presumed RLN injury has a "vocal cord paralysis" simply because the vocal fold appears to demonstrate *motion impairment.* Instead, the laryngologist must use EMG to delineate the exact nature of laryngeal innervation and/or reinnervation, eg, whether synkinesis is present or not. It is beyond the scope of this manuscript to discuss the current state of laryngeal EMG, but increasingly, norms are being developed for the 4 intrinsic muscles, and hence synkinesis (abnormal innervation) is becoming more easily identified. It *is* the intent of this paper to describe the classification of laryngeal synkinesis, as follows.

"*Favorable* synkinesis" (type I synkinesis) is the abnormal innervation of intrinsic muscles, generally with partial or complete motion impairment, but with little or no phonatory or airway deficit or disturbance. In fact, normal phonatory quality is possible.

"*Unfavorable* synkinesis," of which there are 3

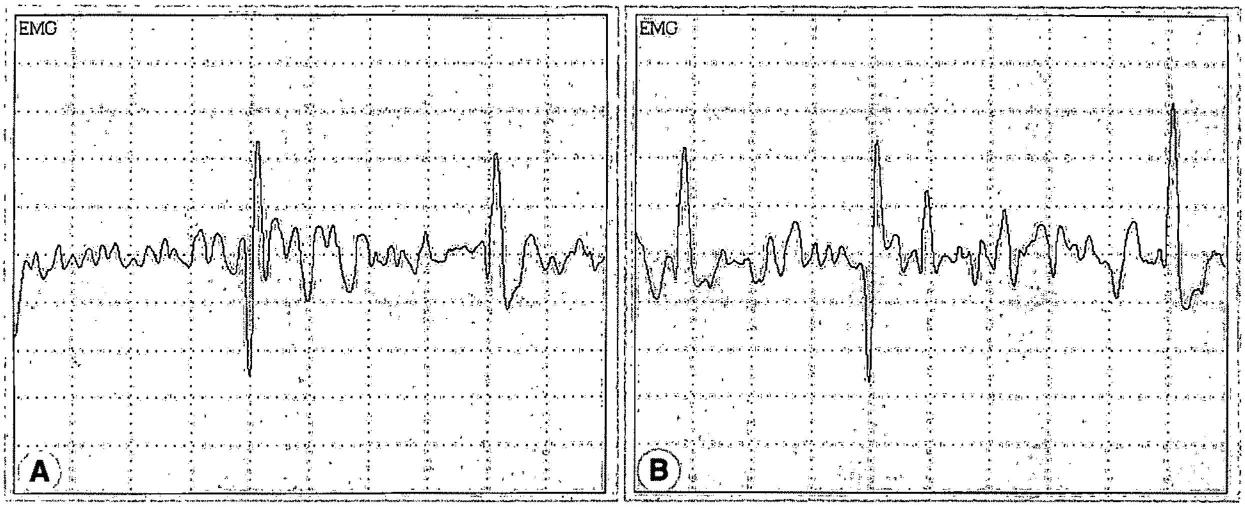

Fig 1. Electromyograms from patient with type I (favorable) synkinesis. Voice was normal, and vocal fold motion absent (thyroarytenoid muscle). A) Phonation of /i/. B) Inspiration.

subtypes, consists of abnormal innervation of intrinsic muscles with motion impairment *and* phonatory and/or airway deficit or disturbance. The 3 subclasses are as follows: spasms, jerking, or twitching of vocal folds, arytenoids, or false vocal folds (type II); hyperadduction (type III); and hyperabduction (type IV).

Accordingly, it is possible to classify synkinesis by recognizing these 4 types of physiological results on the basis of the symptoms and the vocal fold's positioning or behavior, as follows.

TYPE I

Type I laryngeal synkinesis involves an acceptable or normal vocal (phonatory) quality. Because the patient is asymptomatic, he or she may have not seen a physician. Vocal fold motion impairment may be partial or complete. Type I frequently occurs following thyroidectomy and procedures on the cervical spine, particularly when the postoperative voice has been abnormal for a postoperative period of weeks or months but has subsequently improved without treatment. A classic clinical case was presented by Green and Ward[12] (following RLN repair). Type I may be present after ansa cervicalis–RLN anastomosis.[13-15] Electromyography shows inspiratory and phonatory patterns in the TA, LCA, and PCA (Fig 1). No treatment is necessary.

Example. A 44-year-old woman underwent left hemithyroidectomy. Immediately after the operation she had vocal weakness and aspiration. This resolved in 6 weeks, and her aspiration stopped. Gradually, her voice returned to normal. Follow-up laryngoscopy, however, revealed impaired motion of the left vocal fold. An EMG examination revealed both inspiratory and phonatory action potentials in the TA and LCA (Fig 1).

TYPE II

In type II synkinesis, the vocal fold jerks, twitches, or jumps involuntarily. There is often subtle movement of the aryepiglottic fold or false cord. (One should observe the corniculate cartilage position and movement carefully.) The phonatory quality is generally marginal-to-poor. An EMG examination may confirm aberrant reinnervation. The short-term therapy is Botox. For longer-term treatment, ansa cervicalis reinnervation works well (by replacing unfavorable type II synkinesis with favorable type I synkinesis, eliminating vocal fold spasticity).

Example. A 37-year-old woman underwent right thyroid lobectomy. After the operation, her voice was initially weak and subsequently became stronger. However, 6 months after operation it was still somewhat shaky, jittery, and uncontrollable. Fiberoptic laryngoscopy revealed jerky movements of the vocal fold, arytenoid, and aryepiglottic fold. These movements were best seen by observing the corniculate cartilage area. Two years later, the voice had not improved. Ansa-RLN anastomosis quieted the movements and restored her voice. Endoscopically, the spasms and twitching movements were abolished (unfavorable type II converted to favorable type I).

TYPE III

In type III synkinesis, the vocal process and vocal fold are medialized in tonic adduction (relative to the "paramedian" position). Type III is presumably caused by relatively more effective innervation of the LCA compared with the PCA (or any other mechanism that results in the LCA's having stronger contractile properties than the PCA). The phonatory quality is intermediate-to-normal. Airway impairment may be present. For short-term treatment, Botox may be administered to the LCA and/or TA. Longer-

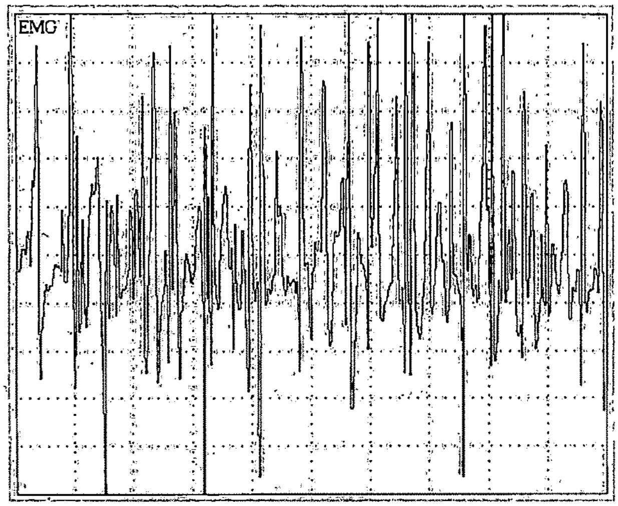

Fig 2. Electromyogram of lateral cricoarytenoid muscle during inspiration demonstrates type III synkinesis. Both thyroarytenoid and lateral cricoarytenoid muscles demonstrated excessive inspiratory activity.

term treatment consists of surgical repositioning of the arytenoid, partial laser arytenoidectomy, or cordotomy. Reinnervation would theoretically be of value in redistributing the balance between the LCA and PCA.

Example. A 53-year-old male physician underwent cervical spine fusion. After the operation, the right vocal cord appeared immobile, and the voice was essentially unusable. The patient underwent Gelfoam injections on 2 occasions. Vocal improvement allowed him to practice medicine. However, at 6 months he began to complain of air hunger and inability to exercise. He also had attacks of severe airway distress. Fiberoptic laryngoscopy revealed a hyperadducted right vocal fold. An EMG examination confirmed excessive muscular activity in the LCA during inspiration (Fig 2). The patient was treated successfully with Botox injections to the right TA and LCA.

TYPE IV

In type IV, the vocal process and vocal fold are lateralized in tonic abduction (relative to the "paramedian" position). Type IV is presumably caused by relatively more effective innervation of the PCA compared with the LCA and IA. (Note that other mechanisms that might evoke stronger muscle contraction of the PCA versus the LCA may be operant. Changes in PCA and LCA muscle fiber types [myosin heavy chain isoforms] may result in imbalance of control for arytenoid abduction versus adduction.[10]) The phonatory quality is theoretically breathy, with a high speech airflow. There is no airway deficit. The author has not seen a perfectly exemplary case; hence, confirmation of type IV (hyperabductive) synkinesis must await EMG evidence of PCA hyperinnervation (in a patient with breathiness and high phonatory airflow rates). The short-term treatment consists of Botox to the PCA. Longer-term treatments include surgical repositioning of the arytenoid, partial resection of the PCA, arytenoid adduction, Isshiki type I thyroplasty, and reinnervation.

Type IV synkinesis is the most probable cause of severe dysphonia and aphonia in patients with vocal fold motion impairment following RLN injury. The cause is hyperinnervation of the PCA with the resultant vocal fold position's being lateral to the usual "paramedian" position. This produces the configuration commonly referred to as an "open posterior commissure." It is usually treated by arytenoid adduction, although reinnervation is frequently successful.

DISCUSSION

Because misdirected regeneration and aberrant reinnervation have long been recognized, there should be no question that laryngeal synkinesis exists. However, the now-antiquated clinical term "vocal cord paralysis" has often been used whenever vocal fold motion impairment follows RLN injury. If "paralysis" means that all 4 ipsilateral intrinsic laryngeal muscles are truly paralyzed (devoid of innervation, denervated), this would appear to be a rare and unlikely scenario. For example, Shindo et al[8] confirmed prior data[16] showing that the canine larynx became reinnervated via RLN regeneration after 3 months, despite removal of a 2.5-cm segment. Other studies[9,14,17-20] have provided a sound basis for the presumption that laryngeal muscles generally regain various levels of innervation following RLN injury. Hence, it appears that aberrant and poorly functioning reinnervation, rather than denervation, is the most common laryngeal problem in patients following RLN injury.

CLINICAL MANIFESTATIONS OF LARYNGEAL SYNKINESIS

Type I. Patients who incur RLN injuries will present the classic clinical signs of unilateral "vocal cord paralysis" in the first few weeks following acute injury. The TA and other laryngeal muscles will undergo gradual diminution in size during this acute period, and clinically, this will be manifest as hoarseness (or even aphonia), excessive phonatory airflow, and aspiration. However, after 3 to 6 months during which RLN axon regeneration has occurred, the clinical results will begin to sort out into 1 of the 4 types of synkinesis. Those patients who are fortunate enough to experience type I synkinesis will demonstrate excellent phonatory quality (by definition). Because they are asymptomatic, they may not even be seen by a laryngologist. (This is not uncommon in patients who have had thyroid surgery performed by a non-otolaryngologist who does not routinely examine the vocal folds via mirror or other laryngoscopy.) Another frequent clinical example is a patient who sustains RLN injury during an orthopedic or neurosurgical operative approach to the cervical spine.

In patients with phonatory weakness or aspiration following such surgical procedures, it is acceptable to follow the patient expectantly if the RLN is thought to be intact. Such a patient should be told that the voice may vary and subsequently revert to normal, and hence a watch-and-wait regimen is justified. Two types of results may follow: 1) neurapraxic RLN fibers will generally regain normal function within 8 to 9 weeks, and normal abduction and adduction will return, or 2) some RLN fibers will undergo axonotmesis or neurotmesis, followed by axon sprouting and regenerative neuronal growth. This is what causes laryngeal synkinesis. If normal function does not return within 2^1/2 to 3 months, it is highly likely that some motion impairment will be permanent.

Temporary Gelfoam injections done in an outpatient setting, such as a voice laboratory or clinic, have been shown to be useful in these scenarios. Because this type I (favorable) synkinesis mimics a normal result in every way (except for vocal fold motion impairment), it is the most desirable result. In fact, for patients with a normal contralateral vocal fold, type I synkinesis is nearly as good as normal, with a potentially normal voice and airway.

Type II. On the other hand, patients with type II synkinesis (spasmodic, jerky vocal folds) generally have moderate-to-severe dysphonia. The phonatory voice may mimic classic adductor spasmodic dysphonia, but more frequently is characterized by excessive air escape and jittery, unpredictable phonatory quality (vocal shakiness, tremor, and unreliability). Such patients will frequently respond to Botox injection of the TA or LCA muscle. Electromyography may help localize the abnormal muscle to be injected, particularly when the action potentials heard and seen via the EMG device coincide synchronously with the observed vocal fold twitches. Another treatment that has proven effective in such cases is ansa cervicalis–RLN anastomosis, which usually converts the spastic type II synkinesis to the more favorable type I, with marked improvement in phonatory quality.[15,21]

Type III. Management of type III synkinesis is more difficult. Fortunately, such cases are also less common. Because the involved vocal fold is hyperadducted, the primary symptom is airway impairment. The vocal quality may be satisfactory-to-normal. Pulmonary studies, such as flow-volume loop analysis, may be useful in quantification of the airway disorder. In addition, magnified laryngeal examination performed in the voice laboratory is often useful. Such high-resolution laryngoscopic imaging will frequently confirm that the vocal fold is not in the classic "paramedian" (slightly lateralized) position, but instead is hyperadducted, close to the midline, or actually in the midline.

One mechanism of airway impairment with type III synkinesis is aberrant reinnervation of the TA by former PCA (inspiratory) axons. This misdirected regeneration produces medial bulging of the vocal fold's middle third during inspiration, an obviously undesirable effect.[22] This may occur *in addition to* hyperadduction (caused by hyperinnervation of the LCA and IA) and may cause air hunger without apparent hyperadduction. Clearly, the best current treatment for this medial bulging phenomenon is EMG-directed Botox injection of the TA. A longer-acting treatment is reinnervation (ansa-RLN anastomosis) to replace the unwanted TA reinnervation with a less-dynamic and more-tonic resting tone associated with the strap muscles (conversion of type III to desirable type I synkinesis).

Type IV. As this classification of synkinesis is physiologically based, it is intuitive that there must be a type IV pattern. If RLN regeneration is truly random, and can cause such undesirable effects as medial bulging from aberrant TA reinnervation, and hyperadduction from LCA and IA hyperinnervation, a mechanism must exist to cause hyperinnervation of the PCA (relative to adductor innervation) and hence *hyperabduction.* A full-blown case of type IV synkinesis would demonstrate vocal fold abduction during attempted phonation. The author has not personally been able to document this fourth pattern as clearly as the first 3. However, it is included herein because it is thought to represent a distinct entity.

Previously, laryngologists were taught that the vocal fold might be "paramedian" in patients with unilateral vocal fold paralysis, and if the superior laryngeal nerve's external branch (to the cricothyroid muscle) were also injured the fold would be "intermediate" in position. The arytenoid and vocal fold *lateralization* seen in the so-called "intermediate" position most likely arises from synkinetic imbalance between the LCA and PCA. In such cases, the PCA has received an inordinate degree of reinnervation relative to the adductor muscles, and the arytenoid is rotated laterally. To make matters worse, it is probable that many of the axons reinnervating the PCA in this scenario are, in fact, formerly adductor axons, producing muscular contraction *during phonation.*

A number of papers have cited the "open" posterior commissure and the problems of abnormal arytenoid abduction following unilateral vocal cord paralysis. If it were not for arytenoid lateral malrotation in such cases, there would be no role for arytenoid adduction procedures; ie, simple Isshiki thyroplasty would provide sufficient medialization of the middle and anterior thirds of the vocal fold. However, it is well known that certain patients need arytenoid adduction to close the posterior commissure. Accordingly, the astute laryngologist will be aware of the hyperabducted arytenoid and the open posterior commissure as evidence of type IV synkinesis. Botox injection of the PCA will allow the IA and LCA to produce some medialization in such cases.

SUMMARY

Patients who sustain RLN injuries demonstrate a wide variability in phonatory and airway results. The 4 classes of synkinesis depicted herein help explain this variation, and provide a physiological basis for the treatment of each type.

There is clearly much to be learned about nerve regeneration and muscle reinnervation. Nerve growth stimulants and inhibitory factors may ultimately be used to induce axons to regrow in more favorable patterns. It is hoped that neuroscientists will soon discover ways to induce abductor axons to sprout back to the PCA while the adductor axons are induced or stimulated to sprout back toward the LCA, TA, and IA.

Until these new scientific breakthroughs occur, the informed laryngologist can do much to help patients with RLN injuries, by recognition of the various types of reinnervation patterns that result in the 4 classes of laryngeal synkinesis.

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SURGICAL PLANNING OF THE TREATMENT OF CHOLESTEATOMA AND POSTOPERATIVE FOLLOW-UP

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There is no single surgical treatment of choice for aural cholesteatoma. The extent of the cholesteatoma, the amount of preoperative destruction, and the size of the mastoid pneumatization should guide the surgeon in choosing the type of operation for a particular ear — which may range from a simple extraction of the cholesteatoma (delivery) all the way to a radical mastoidectomy. It is the clinical acumen that will determine the type of surgery for a given cholesteatomatous ear, algorithms being of little use in the complex situation of a pathological condition with infinite variables. However, even when the most suitable surgical modality is chosen by the best of surgeons, the operated ear will still be left with the innate cause of the cholesteatoma, namely, its physiopathologic background and its tendency to develop a negative gas balance in the middle ear. Because of this tendency to redevelop negative middle ear pressure, insertion and reinsertion of ventilation tubes are often necessary to prevent recurrent retractions and maintain aeration of the middle ear in posterior tympanotomies and modified radical mastoidectomies. Further, when the mastoid bowl is marsupialized, as in radical and modified radical mastoidectomies, the mastoid bowl is often not self-cleansing, thus periodically requiring the help of the otologist to keep it clean and dry. My study consisted of a long-term follow-up of 368 cholesteatomatous ears, which were operated on according to 6 different surgical modalities, ie, 112 radical mastoidectomies, 88 modified radicals, 72 posterior tympanotomies, 52 atticotomies, 36 deliveries, and 8 obliterations. Of the 368 ears, 11% did not require any postoperative toilet, whereas 89% required revisiting the surgeon periodically on an average of every 5 months, for cleansing of the mastoid cavity or securing the aeration of the middle ear by reinserting a ventilation tube.

KEY WORDS — atelectasis, cholesteatoma, eustachian tube, middle ear aeration, retraction pockets, ventilation tubes.

INTRODUCTION

Several surgical modalities have been advocated over the years to treat aural cholesteatoma, each having its ardent advocates. Although recurrence tendency of cholesteatoma is the Achilles' heel of cholesteatoma surgery irrespective of the method used, this matter will not be dealt with in this study, as this was analyzed in detail previously.[1,2] In any case, surgery does not end the saga of the treatment of cholesteatoma, as operated ears require attendance for many years to come. The purpose of this study is twofold: 1) to evaluate the follow-up frequency required of ears operated on by several modalities, in order to keep the surgically exposed mastoid cavity and atticotomy clean and the tympanic cavity aerated in ears that did not undergo a radical mastoidectomy (RM), and 2) to assess the need for postoperative upkeep in the various surgical modalities used in treating cholesteatoma.

MATERIALS

Three hundred sixty-eight (Fig 1) ears with retraction pocket (RP) cholesteatoma treated with 1 of 6 different surgical modalities were followed up for an average of 6.3 years (1.3 to 13.7 years). An RP cholesteatoma was defined as a retraction of the tympanic membrane (TM) into the middle ear (ME) when the accumulating cholesteatomatous debris, mainly keratin, was inaccessible to toilet by suction. Congenital and implantation cholesteatomas were not included in this study.

Most ears were operated on through an endaural incision — the exception being posterior tympanotomy, for which a postauricular incision was most often used.

Radical mastoidectomy was performed in 112 of the 368 (30.4%) patients. Although an ear with an

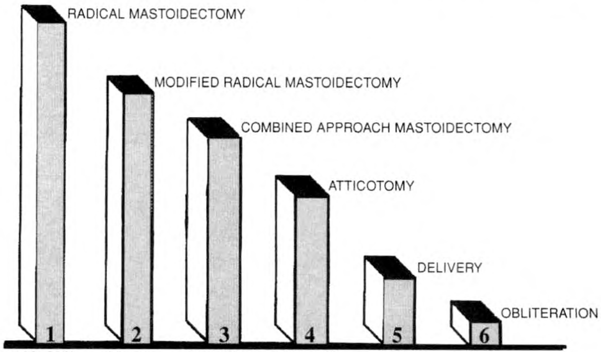

Fig 1. Distribution of various types of surgeries for cholesteatoma.

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RM is considered to be a safe ear, it is well known that the surgical cavity is liable to become uncomfortably wet and infected. In our previous study,[3] we noted that RM cavities were dry when 1) the facial ridge was low, 2) the meatoplasty was adequate, 3) the eustachian tube (ET) was shielded by a buffered air pocket,[4-6] and 4) the mastoid cavity was as small as possible. This led to the concept of the small endaural RM.[7,8] Yet, in spite of satisfactory surgery, most of these ears required a regular periodic cleansing of their cavities. Usually, I perform RM in ears with an extensive cholesteatoma and a small mastoid in which most of the ossicular chain (especially the stapes) is destroyed. However, many of my radical operations have been revisions of imperfect previous surgery, and the cavity was often larger than I wished it to be.

Modified or conservative RMs were performed in 88 (23.9%) patients. These are done when the ossicular chain and TM are intact, the hearing is relatively good and the cholesteatoma is confined mainly to the attic mastoid complex, the TM and ossicular chain can be left intact, hearing can be preserved, and marsupialization can be limited to the attic and mastoid. Although this was Bondy's original concept of the modified RM, today I try to perform this operation in conjunction with a tympanoplasty, even if only a stapes and a remnant of the TM are present. These ears pose 2 postoperative problems: 1) the marsupialized mastoid requires periodic toilet similar to that of the RM, and 2) these ears possess an aerated, often shallow tympanic cavity that may tend to collapse. A collapse can often be avoided by inserting a ventilation tube (VT),[5,6] as originally advocated by Buckingham[9] for atelectatic eardrums.

A posterior tympanotomy and a canal wall–up or combined-approach mastoidectomy were performed in 72 (19.6%) cases. This operation was historically advocated by Jansen[10] and Sheehy and Crabtree,[11] who believed that by extracting the cholesteatoma diligently while at the same time leaving an intact posterior wall, a definite cure of the disease could be achieved. Although removal of the cholesteatoma is often technically feasible, even if the posterior wall is left intact, this operation leaves the ME with its inherent gas economy problem.[12] Indeed, most failures of this operation are due to re-formation of retractions of the TM, which, together with residual cholesteatomas, account for about 60% of failures.[1,2] This can only be observed if a painstaking follow-up is undertaken. Again, retraction of the TM or recurrence of cholesteatoma can be avoided by aeration of the ME with a VT.

Atticotomy was performed in 52 (14.1%) cases. Here, in the presence of a small attic cholesteatoma and a small mastoid, only the attic is marsupialized, leaving an aerated tympanic cavity.

"Delivery" was performed in 36 (9.8%) cases. When the RP cholesteatoma is discovered early and is relatively small and when invasion of the attic and antrum is minimal, the cholesteatoma can often be delivered surgically in its entirety with minimal bone removal, without the need to drill the mastoid. After grafting of the TM, this method will result is an intact ear, but still with its basic gas economy problem, as in atticotomies, conservative RMs, and posterior tympanotomies, and often requires a VT to keep the ME from retracting once again.

Mastoid obliteration was performed in 8 (2.2%) ears. To reduce the postoperative care of the mastoid cavity, Rambo[13] advocated, in "dead ears," obliterating the entire ME cleft and sealing the external ear complex. Later techniques advocated obliterating only the mastoid with soft tissue, bone dust, bone chips, or hydroxyapatite.[14,15] This operation usually leaves an exposed attic, but 2 problems arise. The first concerns the possibility of a cholesteatoma recurrence in the hidden obliterated mastoid — and the mastoid is also "obliterated" to computed tomography. The second is the atrophy of the soft tissue that is used for obliteration.

METHODS

The records of all the 368 patients included in this research were studied. The time between follow-up visits to my office and the state of the ear were noted, especially with respect to the cleanliness of the mastoid and the aeration or collapse of the tympanic cavity. At follow-up, the cavity and VT were cleansed of accumulated debris, and a VT was reinserted into the TM when the VT was found to be extruded and the TM was found to have collapsed again (Fig 2).

RESULTS

Marsupialized attics and mastoid cavities comprised 112 exposed cavities with RM and 88 cases of modified and radical cavities, as well as 52 exposed attics that behaved like very small cavities, for a total of 252 (68.5%) of the entire series (Fig 3).

Twelve (3.3%) of the entire series or 4.7% of the mastoid cavities were practically always somewhat moist, even if cleaned frequently. The discharge usually came from the exposed part of the ET (in RM) or from mucosal metaplastic patches in the mastoid bowl (usually in a cellular or diploic mastoid). While these cavities were not, as a rule, completely dry, they were in a tolerable state if cleaned on the average once every 2 months. When cleansing was ne-

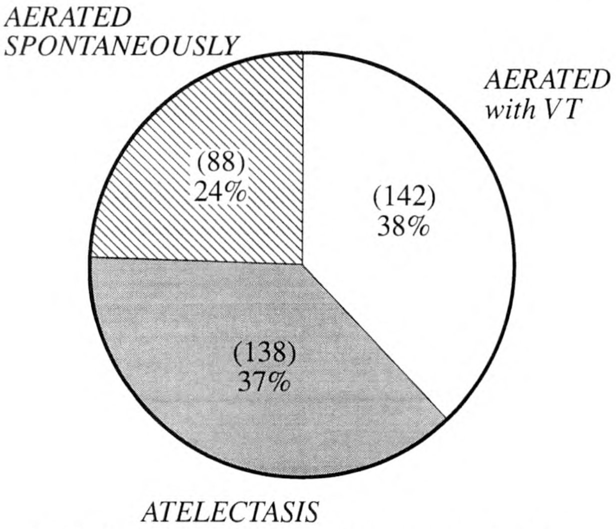

Fig 2. Percentages of 368 ears that were aerated spontaneously postoperatively (24%), that required ventilation tube (VT; 38%), and in which tympanic membrane totally collapsed (37%).

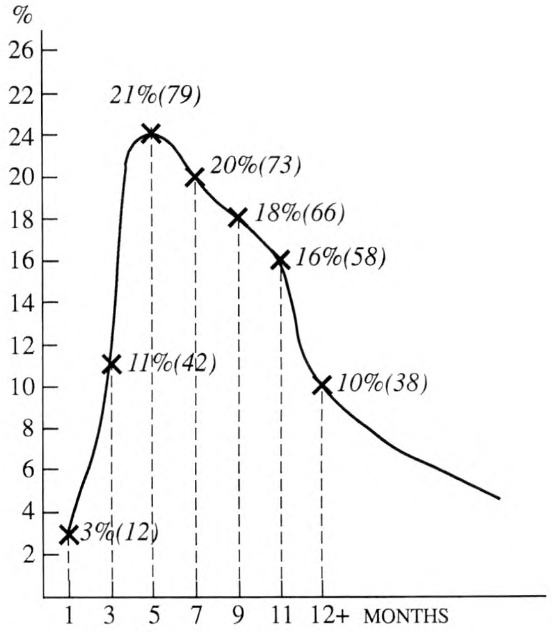

Fig 3. Average frequency of postoperative visits (368 ears).

glected, the debris that formed became infected, occasionally leading to the formation of granulation tissue and polyps; ie, they turned into running ears. One of my patients was a physician who had had bilateral RMs. Both cavities presented a low facial ridge, the meatoplasty was adequate, and the mastoid was of medium size — yet, according to the patient, the cavities produced up to 15 mL of mucopurulent discharge per day. This discharge was seen to come from a thick infected mucosa and granulation tissue, which occurred because the ears were unattended. Both ears dried up after 2 outpatient treatments.

Thirty-eight (10.3%) cavities were always dry, requiring no treatment after surgery. They were covered by a dry epithelium. The debris formed cleared completely and spontaneously, and very little keratin was formed altogether. When the ears were examined, once every several years, hardly any debris was found that required removal.

The remaining 318 (86.4%) ears had 202 cavities that filled up with debris and needed cleansing on an average of every 5 months (Fig 3) in order to remain dry. The smaller the cavity, the longer the interval between follow-up visits. The decision as to how often a particular ear required cleansing was clinically determined during the follow-up period by myself and the patients. If an appointment was long overdue, the debris in the cavity accumulated and at times became moist and infected. This usually happened when the patient was unaware of the accumulating debris.

As noted in Fig 2, 230 (62.5%) ears presented an aerated ME cavity, of which 88 (23.9%) were self-sustaining aerated MEs, while 142 (38.6%) required a VT to aerate them. The other 138 (37.5%) presented no air cavity.

A VT was inserted either at primary surgery or at some time during follow-up when the TM was seen to retract, and it stayed in place for 5 months on average. Eleven ears required a T-tube because they extruded their VTs rapidly, within 2 months after insertion. Once the VT was extruded, some ears stayed spontaneously ventilated, whereas in others, the TM collapsed and a new VT was required. A VT can be inserted quite easily with the help of local anesthesia, ie, a drop of phenol. The time span of re-formation of an atelectasis was between 2 days and 8 weeks.[16] It should be noted that although the VT was an effective ventilator in most ears, 6 ears with posterior tympanotomies (1.5% of the aerated ears) showed a collapse or a retraction of the TM, sometimes into the mastoid, despite the presence of a patent VT.

DISCUSSION

A regular RP can be cleaned of accumulating debris by suction, and the RP can often be corrected with the aid of a VT, unless the RP is already adherent to the medial surface of the mesotympanum. Such RPs can also be excised and the defect grafted; however, a VT will often be required to prevent recurrent retraction. Once the RP is deep and cannot be cleared by suction, it turns into an RP cholesteatoma. The background of RP cholesteatomas, which constitute about 90%[4] of all cholesteatomas (other types being implantation, invasive, "congenital," and metaplastic cholesteatomas), is a disorder of ME gas economy[12] leading to a negative pressure, which usually occurs in ears whose mastoid is hypopneumatized.[4,16,17] The consequence of retraction of any part of the TM is usually a deficient clearance of keratin and dead cells, which usually become infected secondarily. Pressure of the sac and the accompanying inflammatory process will eventually lead to destruction of the ossicular chain and other ME structures, resulting in hearing loss and a discharging ear.[18-20] The important classic intracranial complications, which may also ensue, are rare today in Western societies, probably because of early detection and prompt treatment.

The goal of treatment of cholesteatoma is to stop its destructive activities, ie, remove the cause and repair, when possible, the damage that has occurred to the ossicles and TM and prevent recurrence. However, the concept that diligent removal of cholesteatoma and reconstruction of destroyed structures will solve the problem completely does not take into account that even the best surgery will not remove the pathogenetic background that led to the cholesteatoma in the first place, ie, the tendency to retract, nor the new problems arising from surgery itself, such as the creation of a marsupialized mastoid bowl with its inherent problems. Although the main problem can be managed and solved in a certain number of ears with one surgical modality or another, most ears will require periodic rechecking to ensure a dry ear and to discover early retractions and recurrences.[1,2]

Various surgical methods have been advocated for the treatment of cholesteatoma. Each method has its ardent advocates as being the treatment of choice as far as the long-term results are concerned. However, this holistic approach is an oversimplified reaction to a complex situation, because cholesteatomas do not have a uniform presentation nor a uniform postoperative course. In fact, in order to obtain the best results for a given ear, one should tailor a specific surgical modality to each specific ear, according to the special features at hand. The main factors in question are 1) the size, location, and depth of the lesion, 2) the type and size of mastoid pneumatization, and 3) the amount of destruction caused to the ME structures and to hearing. Nevertheless, all types of surgery will leave the ear with problems as far as its future is concerned. Thus, radical and modified mastoidectomies will often leave a marsupialized mastoid bowl and atticotomies will leave an exposed attic, all with their potential clearance problems. Modified RMs and atticotomies will leave both a mastoid bowl and a small aerated tympanic cavity. Other methods, such as posterior tympanotomies and "delivery," will leave both an aerated tympanic cavity and an aerated mastoid bowl. Any aerated part of the ME is liable to retract, despite removing the cholesteatomatous process as completely as possible intraoperatively with the operating microscope. Thus, the cholesteatoma may return either in the form of growing pearls or as a flat growth of squamous epithelium. The origin of the reappearance of squamous epithelium may be some unobserved residual epithelium, due to a metaplastic process[21] or a recurrent retraction of the TM into a potential dead space. This latter form is the most frequent cause for surgical failure.[2,5]

A marsupialized part of the ME cleft, attic, or mastoid is ideally covered by squamous epithelium, which will synthesize keratin the same as the TM or atelectatic TM.[20] Some mastoid cavities are lined by an epithelium that synthesizes small amounts of keratin; others synthesize a great deal of keratin. In some, the keratin is soft, and in others, firm. However, some mastoid bowls are not lined by stratified squamous epithelium, and others are wet from mucus spilling over from an exposed ET.

When a minute foreign body (charcoal) is placed in the tympanic opening of an exposed ET, it may stay there for days, weeks, and even months without being cleared by the physiological mucociliary system, in contrast to what happens in simple chronic otitis media, in which the small bits of foreign body disappear into the lumen of the ET.[21,22] Cholesteatomas seem to have a double clearance failure: one concerning debris from the retracted sac or mastoid bowl, and the other, mucus production from the ET. As are other pathological processes in which infection is secondary to dead organic material isolated from the circulation, infections that occur in a mastoid bowl filled with necrotic epithelial debris are usually resistant to antibiotic therapy. The basic treatment of both infected and noninfected debris is cleaning the cavity by suction curettage, etc, with the aid of the operating microscope. Our present study shows that to keep a mastoid bowl clean, most ears require a periodic toilet averaging once every 5 months (Fig 3).

The second inherent postoperative problem regarding cholesteatoma surgery relates to aeration of the tympanic cavity. Aeration is spontaneous in 24% (Fig 2), but 38% require the assistance of a VT. In our material, the average duration of a VT in situ was circa 5 months. Ventilation tubes, especially T-tubes, accumulate debris around themselves that also requires cleansing; otherwise, infection and even granulation tissue may develop. Once the VT has been extruded, some tympanic cavities aerate spontaneously; others show a recurrence of the atelectasis or re-formation of the retraction pocket, which demands reinsertion of a VT.

In addition to debris accumulation, infection, and collapse of the TM, there were other postoperative problems requiring treatment. Three of my patients had a tendency to develop a stubborn fungal infection in their mastoid bowl. Seven patients showed a spontaneous, partial formation of soft tissue membrane at the site from which the posterior wall was removed, despite the fact that the posterior wall was removed down to the facial nerve. One patient developed a "blue cyst" in the mastoid bowl, which was an encapsulated cholesterol granuloma. Several patients developed acute and secretory otitis media in an aerated tympanic cavity, in the absence of a VT, and 4 children developed acute mastoiditis long after a successful posterior tympanotomy.[23]

CONCLUSIONS

The main conclusion from this study is that follow-up is mandatory for all patients who undergo operation for cholesteatoma. This is a tedious, time-consuming effort, both for the surgeon and for the patient. In the absence of such follow-up, an unrealistically optimistic attitude toward the future well-being of operated cholesteatomatous ears may develop. It is also of prime importance to inform the patients before surgery as to what to expect from the operation and the ensuing years.

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MIGRAINE AND BENIGN POSITIONAL VERTIGO

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Because inner ear symptoms are common in patients with migraine, we questioned whether benign positional vertigo (BPV) is more common in patients with migraine than in the general population. We reviewed the records of 247 patients seen in our neurotology clinic over the past 5 years with a confirmed diagnosis of BPV. Each patient had the typical history of BPV, and in each case the characteristic torsional vertical positioning nystagmus was identified. All were interviewed regarding migraine symptoms by means of standard International Headache Society criteria. Migraine was 3 times more common in patients with BPV of unknown cause than in those with BPV secondary to trauma or surgical procedures. Most patients were cured with the particle repositioning maneuver, regardless of the cause. Presumably, patients with migraine suffer recurrent damage to the inner ear (due to vasospasm or some other mechanism) that predisposes them to recurrent bouts of BPV.

KEY WORDS — migraine, positional nystagmus, vertigo.

INTRODUCTION

Dizziness is a common complaint in patients with migraine. About two thirds of patients report motion sensitivity with frequent bouts of motion sickness, and about a fourth experience discrete spells of vertigo.[1,2] Migraine can mimic Meniere's disease, and so-called "vestibular Meniere's disease" is often a migraine equivalent.[3] Of all of the vertigo syndromes, the most common is benign positional vertigo (BPV). It occurs when freely floating otoconia normally attached to the utricular macula enter the posterior semicircular canal and move under the influence of gravity. Although BPV can occur after head trauma, it is most commonly seen in older patients without any apparent cause.[4,5] We recently reported 3 members of a family with childhood-onset BPV, each of whom also had migraine.[6] Because BPV is so rare in children, we suspected that it was part of a migraine syndrome in this family. To assess the relationship between migraine and BPV in a larger patient population, we reviewed the records of 247 consecutive patients seen by the senior investigator (R.W.B.) in the preceding 5 years who had a confirmed diagnosis of BPV.

METHODS

We reviewed the records of 247 patients who met the following criteria: 1) a history of brief episodes of positional vertigo typical of BPV and 2) a characteristic torsional vertical paroxysmal positioning nystagmus on examination. The nystagmus was induced by a rapid change from the sitting position to the head-hanging right and/or head-hanging left positions (the Dix-Hallpike test). Patients who reported a typical history suggestive of BPV but who did not exhibit the characteristic positioning nystagmus on examination were not included. Also, patients who exhibited other types of positioning nystagmus, including the horizontal and anterior canal variants and central variants, were excluded.

Each patient completed a detailed questionnaire summarizing the key features of his or her clinical history. The patient was then interviewed and examined. Criteria of the International Headache Society were used for the diagnosis of migraine (Table 1[7]). The age of onset of BPV was based on the patient's report of the initial occurrence of BPV. (If they had prior bouts followed by remissions, the onset was set at the beginning of the initial bout.) The BPV

TABLE 1. INTERNATIONAL HEADACHE SOCIETY DIAGNOSTIC CRITERIA FOR MIGRAINE HEADACHES

A. At least 5 attacks fulfilling B-D
B. Headaches last 4-72 hours (untreated)
C. Headache has at least 2 of following features:
    Unilateral
    Pulsating
    Moderate to severe (inhibits or prohibits daily activities)
    Aggravated by walking, stairs, or similar physical activities
D. During headache, at least 1 of following:
    Nausea and vomiting
    Photophobia and phonophobia
E. Other causes for headache have been ruled out

Information from Olesen.[7]

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TABLE 2. FREQUENCY OF MIGRAINE IN PATIENTS WITH BENIGN POSITIONAL VERTIGO OF KNOWN VERSUS UNKNOWN CAUSE

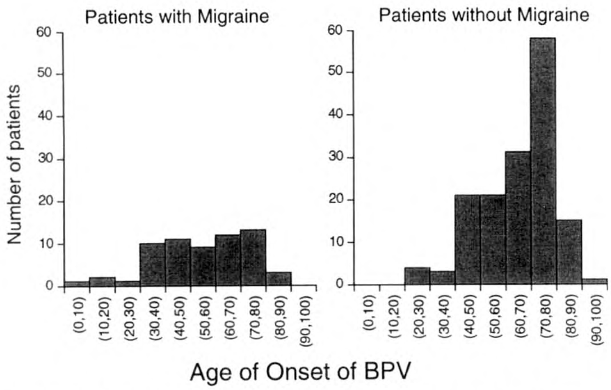
	All		*Women*		*Men*	
Cause	*No.*	*%*	*No.*	*%*	*No.*	*%*
Trauma or surgery	3/31	10	2/15	13	1/16	6
No known cause	62/216	29	48/139	35	14/77	18
Totals	65/247	26	50/154	32	15/93	16

was considered recurrent if it recurred after a spontaneous remission (by history) or if it recurred after the particle repositioning maneuver (by history and examination).

All patients underwent a modified Epley maneuver for treatment of their BPV.[8,9] The maneuver was repeated until the positional nystagmus and vertigo disappeared (up to 4 times) or until the patient was unable to continue because of nausea. Patients were instructed to avoid lying flat for 48 hours to prevent the debris from reentering the canal. They were told to call in a week if they continued to have positional vertigo. The maneuver was considered successful if no nystagmus was observed in any of the positions during the final maneuver and the patient did not call to report a recurrence. Instances in which the nystagmus and vertigo were diminished but not absent were considered unsuccessful treatments. Also, if the patient was unable to complete the maneuver because of nausea, it was considered unsuccessful. Typically, the latter patients were rescheduled for another visit on which they were premedicated before the maneuver was retried.

RESULTS

Of the 247 cases that met the entry criteria, BPV was secondary to head trauma in 21 and occurred in the immediate postoperative period after a variety of surgeries in 10. Because it is unlikely that migraine was involved in the pathophysiology of BPV in these 31 cases, they provided a good reference group for the incidence of migraine in our population. The incidence of migraine in the remaining patients with BPV was 3 times the incidence in patients with an identifiable cause (Table 2). Further, the distribution of the age of onset of BPV was different in those with migraine and those without migraine (see Figure). The age of onset in patients with BPV and migraine was distributed rather evenly over multiple decades, whereas the age of onset in patients with BPV and no migraine was skewed toward the older ages, with a peak in the eighth decade. About half (47%) of the patients with onset of BPV before the age of 50 years had migraine. By contrast, only 15% of patients with onset of BPV after the age of 50 years had migraine.



Distribution in age of onset of benign positional vertigo (BPV) in patients with and without migraine.

TABLE 3. PERCENTAGES OF PATIENTS WITH BILATERAL, RECURRENT, AND SUCCESSFULLY TREATED BENIGN POSITIONAL VERTIGO

			Idiopathic	
	All (*n = 247*)	*Trauma or Surgery* (*n = 31*)	*Migraine* (*n = 62*)	*Non-migraine* (*n = 154*)
Bilateral	8	16	5	7
Recurrent	68	61	77	66
Successful treatment	86	74	82	87

Surprisingly, there was a high recurrence rate of BPV in all categories (Table 3). The highest recurrence rate occurred in patients with migraine. Seventy-seven percent of the patients with BPV and migraine had at least 2 separate bouts of BPV (before or after treatment). The particle repositioning maneuver was highly successful in all groups, with an overall cure rate of 86%. A lower cure rate in the trauma-postsurgical group can be traced to the fact that bilateral BPV (simultaneous in both ears) was common in the posttraumatic group (6 of 21 cases), and only 1 of these 6 cases of bilateral BPV was cured after a single session. Bilateral BPV was infrequent in the migraine and nonmigraine idiopathic groups. Thirty-one patients in the idiopathic group (14%) reported a prior bout of spontaneous vertigo before the onset of BPV. Of this group, 13 (42%) had migraine.

DISCUSSION

We found that patients with BPV of unknown cause were 3 times more likely to have migraine than patients with BPV secondary to a likely cause (head trauma or surgery). Because migraine and BPV are so common, both diagnoses could occur in the same patient on the basis of chance alone. However, we do not think that the high incidence of migraine in our BPV population is a chance occurrence, because the incidence in patients with BPV secondary to trauma or surgery was similar to that in the general population.[10] Also, the age of onset in patients with BPV and migraine was different from that in patients with BPV without migraine. Almost half of the patients with onset of BPV under the age of 50 years met the diagnostic criteria for migraine. Only 15% of the patients with onset of BPV after the age of 50 years had migraine.

The mechanism for the vestibular symptoms and signs associated with migraine is poorly understood.[11] Vasospasm of the labyrinthine arteries is a possible mechanism, because vasospasm is a well-documented phenomenon with migraine. Monocular visual loss caused by vasospasm of the ophthalmic artery occurs with migraine, and episodes of monocular visual loss can be prevented with antispasmodic agents.[12] Benign positional vertigo is a well-documented sequela to ischemic damage of the inner ear, presumably due to release of otoconia from the macular membrane.[13] Although vasospasm definitely occurs with migraine, it may be a secondary phenomenon due to some underlying metabolic disorder. The vasospasm associated with the classic visual aura is almost certainly secondary to a primary neuronal metabolic defect (the so-called "spreading wave of cortical depression").[14] Vasospasm in the inner ear might also be secondary to a primary metabolic abnormality in the inner ear. Peripheral vestibular abnormalities seen on electronystagmographic examination are more common in patients with migraine than in control groups.[11,15]

Recently, some families with hemiplegic migraine were found to have mutations in the gene coding for the α1A transmembrane subunit of a voltage-gated calcium channel.[16] This gene is one of a large family of genes that code for different subunits of voltage-gated neuronal calcium channels, several of which are expressed in hair cells and neurons. In the inner ear, ion channels are critical for maintaining resting potentials and excitation of the primary afferent neurons. Mutations in ion channel genes shared by the brain and inner ear could explain the brain and inner ear symptoms in our patients with migraine and BPV.

A consistent unexplained feature of BPV is the female preponderance of about 2:1 in idiopathic cases.[4,5] Because migraine is 2 to 3 times more common in women than men, a migraine cause for BPV could at least in part explain the female preponderance. There is relatively little information in the literature regarding the recurrence rate of BPV, although more recent publications have emphasized that recurrences are common. Overall, about two thirds of our patients with BPV either reported prior bouts of BPV or had recurrences of BPV during follow-up. More than three quarters of the patients with migraine and BPV had recurrent bouts of BPV. It is unclear why some patients have multiple recurrences whereas others have only a single bout.[17] The otoconial debris may dissolve in the endolymphatic fluid or may be phagocytosed into the wall of the membranous labyrinth. Presumably, patients with migraine suffer recurrent damage to the inner ear (due to vasospasm or some other mechanism) that predisposes them to recurrent bouts of BPV. In patients with frequent recurrences of BPV, we recommend that they avoid activities involving extreme backward extension of the head (eg, leaning backward into a hairdresser's sink, certain physical exercises, working under an automobile, etc).

The particle repositioning maneuver had a high success rate regardless of the cause of BPV.[18] This would suggest that regardless of what damages the inner ear, the canalithiasis mechanism accounts for the BPV. In the past, we and others concluded that patients who reported a prior spontaneous attack of vertigo followed years later by BPV probably initially had a viral vestibular neuritis.[4] However, in the present study, 42% of the 31 patients who reported a prior prolonged attack of vertigo met the diagnostic criteria for migraine. This finding suggests that some of these earlier vertigo attacks may have been migraine equivalents rather than a viral inner ear disorder.

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CORRELATION OF PURE TONE AUDIOGRAM RESULTS AND HEARING BENEFIT OF TYMPANOPLASTY FOR CHRONIC SUPPURATIVE OTITIS MEDIA

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The aim of this study was to evaluate the correlation between pure tone audiogram results and the subjective sensation of hearing benefit of patients who had tympanoplasty for chronic suppurative otitis media. This is a prospective study of 115 patients who had tympanoplasty between 1992 and 1994. The outcome, including a pure tone audiogram and the subjective sensation of hearing benefit, was evaluated at 1 year after operation. There were 63 (55%) patients with subjective hearing benefit after the tympanoplasty. The subjective sensation of hearing benefit correlated with the magnitude of the air conduction (AC) threshold reduction, and increased from 39% for an AC reduction of less than or equal to 10 dB to 100% for an AC reduction of more than 30 dB. The effect of the interaural AC threshold difference on the subjective sensation of hearing was not significant; improvement was felt by 92% of patients when the operated ear became the better hearing ear, and by 73% of patients when the operated ear remained the worse hearing ear. There was considerable discrepancy between the subjective hearing benefit and the pure tone audiogram results. A combination of parameters, including the air-bone gap, the AC threshold, and the subjective hearing change, is recommended in reporting the results of tympanoplasty.

KEY WORDS — hearing, otitis media, tympanoplasty.

INTRODUCTION

Tympanoplasty for chronic suppurative otitis media is indicated mainly for the eradication of suppurative disease to achieve a dry ear. The criteria for successful eradication of suppurative otitis media can be clearly defined by the presence of an intact eardrum and a dry ear. The results are well documented in the literature.

Hearing benefit is also desirable after successful eradication of the underlying suppurative otitis media. There are, however, no unanimous criteria of successful hearing results after tympanoplasty.[1-7] The commonly used measures mainly take into consideration the technical aspects of the operation, including the closure of the air-bone (AB) gap and the reduction of the air conduction (AC) threshold. There is controversy as to how the AB gap and AC shift should be reported, and guidelines have been recommended on a uniform reporting system by the Committee on Hearing and Equilibrium of the American Academy of Otolaryngology–Head and Neck Surgery.[7] The guidelines, however, still concentrate on the technical results rather than the functional benefit to the patient.

From the patient's point of view, the subjective sensation of hearing benefit is more realistic and may not be directly reflected by the degree of AB gap closure or AC threshold reduction. Because the ultimate aim of surgical repair of the conductive mechanism is improvement of the subjective sensation of sound and speech, information on the magnitude of a discrepancy or correlation between the pure tone audiogram results and the subjective hearing benefit of tympanoplasty is important for the surgeon and the patient. In the present study, we analyzed the correlation between pure tone audiogram results and the subjective hearing benefit to the patient. We hope the results will enable both patients and surgeons to understand the hearing benefit and will help in counseling before middle ear surgery.

METHODS AND PATIENTS

This is a prospective study of the hearing results of tympanoplasties for chronic suppurative otitis media performed from January 1992 to December 1994 in the Division of Otorhinolaryngology, Queen Mary Hospital, Hong Kong. Patients were included only if the operation had achieved a dry ear with eradication of the suppurative otitis media at the end of 1 year after operation. Patients who had radical mastoidectomy or persistent or recurrent tympanic mem-

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TABLE 1. RELATIONSHIP OF PREOPERATIVE AND POSTOPERATIVE AIR-BONE GAPS

Preoperative Air-Bone Gap (dB)	*No. of Pts*	*Postoperative Air-Bone Gap (dB)* *≤10* *No.*	*%*	*11-20* *No.*	*%*	*21-30* *No.*	*%*	*>30* *No.*	*%*
≤10	10	9	90	1	10				
11-20	38	19	50	16	42	2	5	1	3
21-30	40	14	35	14	35	9	23	3	8
31-40	17	2	12	6	35	2	12	7	41
41-50	10	1	10	4	40	1	10	4	40
Total	115	45	39	41	36	14	12	15	13

TABLE 3. RELATIONSHIP OF PREOPERATIVE AIR-BONE GAP TO POSTOPERATIVE SUBJECTIVE HEARING CHANGE

Preoperative Air-Bone Gap (dB)	*No. of Pts*	*Postoperative Subjective Hearing Change* *Improved* *No.*	*%*	*NS* *No.*	*%*	*Worse* *No.*	*%*
≤10	10	6	60	4	40	0	
11-20	38	21	55	17	45	0	
21-30	40	22	55	15	38	3	8
31-40	17	8	47	7	41	2	12
41-50	10	6	60	4	40	0	
Total	115	63	55	47	41	5	4

NS — no significant change.

brane perforation within the first year after tympanoplasty, or with postoperative follow-up of less than 1 year, were excluded. There were 115 patients included in the present analysis. The indications for operation on these patients included 89 central perforations, 3 marginal perforations, 6 retraction pockets, and 17 marginal perforations with cholesteatoma or retraction pockets with cholesteatoma. The operations were 81 myringoplasties alone, 8 myringoplasties with ossiculoplasty, and 26 mastoidectomies (19 modified radical mastoidectomies, 6 intact canal wall mastoidectomies, 1 atticotomy), with 9 of them having 1-stage ossiculoplasties.

The outcome evaluation was made 1 year after operation. All patients had a pure tone audiogram before and at the end of the first year after the operation. The patients were asked whether the operation had resulted in any significant hearing benefit in their daily life, and they chose 1 of 3 subjective sensations: 1) improved, 2) no significant change, or 3) worse. The air and bone conduction thresholds at 0.5, 1, 2, and 4 kHz were used in the calculation of mean AB gap and AC threshold shift.

RESULTS

There were 115 patients (49 male and 66 female). The median age was 40 years (range 16 to 75 years).

The relationship of the preoperative AB gap to the postoperative AB gap is shown in Table 1. Of the 67 patients who had a preoperative AB gap of more than 20 dB, 17 (25%) patients had a postoperative AB gap within 10 dB and 41 (61%) patients had a postoperative AB gap within 20 dB. Of the 48 patients who had a preoperative AB gap within 20 dB, 45 (94%) patients had a postoperative AB gap maintained within 20 dB.

The relationship of the preoperative AB gap to the postoperative AC threshold change is shown in Table 2. Of the 105 patients who had a preoperative AB gap of more than 10 dB, 50 (48%) patients had a postoperative AC threshold reduction of at least 10 dB. Of all 115 patients, 3 (3%) patients had a deterioration of the postoperative AC threshold of at least 10 dB.

The relationship of the preoperative AB gap to the postoperative subjective hearing change is shown in Table 3. There were 55% patients who subjectively felt a significant improvement of hearing after the operation. The chance of hearing improvement was within a narrow range, between 47% and 60%, irrespective of the magnitude of the preoperative AB gap.

The relationship of AC threshold reduction and subjective hearing change is shown in Table 4. The incidence of subjective hearing improvement increased with increasing magnitude of AC reduction, and was 100% when there was a 30-dB or more AC reduction (Mantel-Haenszel test for trend, p = .0018).

TABLE 2. RELATIONSHIP OF PREOPERATIVE AIR-BONE GAP TO POSTOPERATIVE AC THRESHOLD CHANGE

Preoperative Air-Bone Gap (dB)	*No. of Pts*	*Postoperative AC Threshold Change (dB)* *↓31-40* *No.*	*%*	*↓21-30* *No.*	*%*	*↓11-20* *No.*	*%*	*≤10* *No.*	*%*	*↑11-20* *No.*	*%*	*↑21-30* *No.*	*%*
≤10	10							10	100				
11-20	38			3	8	11	29	23	61	1	3		
21-30	40	2	5	2	5	16	40	19	48			1	3
31-40	17	1	6	4	24	4	24	7	41	1	6		
41-50	10	3	30	2	20	2	20	3	30				
Total	115	6	5	11	10	33	29	62	53	2	2	1	1

AC — air conduction; ↓ — decreased AC threshold; ↑ — increased AC threshold.

TABLE 4. RELATIONSHIP OF POSTOPERATIVE AC THRESHOLD REDUCTION TO SUBJECTIVE HEARING IMPROVEMENT

Postoperative AC Reduction (dB)	No. of Pts	Patients With Subjective Improvement No.	%
1-10	36	14	39
11-20	33	24	73
21-30	11	10	91
31-40	6	6	100
Total	86	54	63

The effect of the interaural AC threshold difference on the subjective hearing improvement of patients who had more than a 10-dB reduction of AC is shown in Table 5. Patients with a postoperative AC threshold better than that in the contralateral ear had a higher incidence (92%) of subjective hearing improvement; however, the trend is not statistically significant (Mantel-Haenszel test for trend, p = .433).

DISCUSSION

The primary aim of middle ear surgery for chronic suppurative otitis media is the eradication of the suppurative condition to achieve a dry ear. In those patients who have achieved the primary aim, hearing benefit is also desirable. The AB gap and AC threshold are frequently used as the only outcome indicators of the hearing results of tympanoplasty. It is, however, unknown as to how much of this AB gap closure or AC reduction can be translated into a patient's subjective improvement of hearing. For those patients who had successfully achieved a dry ear with an intact tympanic membrane, the present study indicated that there were considerable discrepancies between the surgeons' viewpoint of success based on pure tone audiogram results and the patients' subjective sensation of hearing improvement.

Air-bone gap closure is frequently used as the criterion of successful hearing results of tympanoplasty. Of those patients who started with a minimal conductive hearing loss with a small AB gap of less than 20 dB, 94% of patients continued to have a small AB gap after the operation. It showed that there was a high success rate of preservation of the conductive pathway. Of the patients who started with a significant conductive hearing loss, with a large AB gap of more than 20 dB, the prognosis of closure of the AB gap to within 10 dB was 25%, and to within 20 dB was 61%. The chance of AB gap closure, however, decreased with increasing magnitude of the preoperative AB gap, as shown in Table 1. The magnitude of the preoperative AB gap reflected the extent of damage to the conductive pathway. It showed that the more damage to the conductive mechanism, the more difficult it was to repair completely. Although closure of the AB gap is frequently used in reporting results, it reflects more on the postoperative residual conductive defect than on the magnitude of correction of the defect. For instance, a procedure with a preoperative AB gap of 30 dB and postoperative reduction of the AB gap to 20 dB will be considered successful by a surgeon and is easily achieved with more than a 61% chance; however, it only improves the AC threshold by 10 dB, and the chance of subjective improvement of hearing is only 39%. Although the AB gap is a meaningful outcome measure of surgical success, it is not directly correlated with the patient's subjective sensation of hearing benefit.

TABLE 5. EFFECT OF INTERAURAL DIFFERENCE OF AC THRESHOLD ON SUBJECTIVE HEARING BENEFIT OF PATIENTS WITH MORE THAN 10-DECIBEL AC THRESHOLD REDUCTION

AC Threshold of Operated Ear Compared With That of Contralateral Ear	No. of Pts	Patients With Subjective Improvement No.	%
Operated ear better, difference >10 dB	12	11	92
Interaural difference within 10 dB	23	18	78
Operated ear worse, difference >10 dB	15	11	73

Air conduction threshold reduction is also commonly used in reporting tympanoplasty results. The AC threshold change reflects more on the magnitude of the surgical correction of the damaged conductive pathway than on the residual conductive defect, as in the AB gap. The overall prognosis of an AC reduction of more than 10 dB was 48% after tympanoplasty. The magnitude of the postoperative AC reduction increased with an increasing preoperative AB gap. It showed that although it was more difficult to completely correct the conductive defect in patients with a large preoperative AB gap, there was, however, a larger magnitude of reduction of the AC threshold in these patients, which implied a higher chance of some degree of subjective improvement of hearing. For instance, a patient with a preoperative AB gap of 55 dB and a postoperative reduction of the AB gap to 25 dB will be considered a failure of surgery by the AB gap closure criterion; the magnitude of the AC threshold reduction by 30 dB, however, means a 100% chance of subjective hearing benefit to the patient.

Subjective hearing benefit is seldom used nowadays in reporting the results of tympanoplasty. It is, however, the most important criterion for the justification of surgical success by the patient. The ultimate goal of tympanoplasty is to improve the subjective perception of sound and speech. Therefore, it should be included in reporting the hearing results of middle ear surgery. Our results showed that the overall chance of a subjective hearing benefit was 55%, and this did not correlate with the preoperative AB gap size. The results also showed that the larger the magnitude of the AC threshold reduction, the higher the chance of having a significant subjective improvement of hearing. The chance of subjective improvement increased progressively from 39% to 100% when the AC threshold reduction increased from less than 10 dB to over 30 dB. Objective improvement of the AC threshold did not necessarily result in a subjective hearing improvement, unless the AC reduction was larger than 30 dB. A reduction of the AC threshold of less than 30 dB might still be meaningful for the patient, but the prognosis of subjective hearing benefit decreased progressively with decreasing AC threshold reduction.

The interaural AC threshold difference has been suggested to influence the hearing benefit.[1,2,6] Of those patients who had a more than 10-dB reduction of the AC threshold, there was only a slightly higher chance of a subjective hearing improvement if the operated ear became the better hearing ear. On the other hand, despite the operated ear's remaining the worse hearing ear, over 70% of patients still benefited from the AC threshold reduction. Therefore, the hearing result of the operated ear is still the most important factor contributing to the hearing benefit of the patient; the influence of the contralateral ear should not be overemphasized in the hearing benefit.

In preoperative counseling of patients, apart from advice on the prognosis of achieving an intact tympanic membrane with a dry ear, the prognosis of hearing results in terms of AB gap closure and AC reduction and their significance on subjective hearing benefit should also be given. The data in Tables 1-5 are helpful in the preoperative counseling of patients as to their prognosis of both objective and subjective hearing benefits when the suppurative otitis media can be eradicated successfully.

The reporting of surgical results and the preoperative counseling of patients should take into consideration both subjective and objective hearing results. The weak point of subjective evaluation of a hearing change is the difficulty of quantification. In the present study, we can only quantify subjective hearing improvement as "improved," "no significant change," or "worse." This quantitative method is relatively simple and easily understood by the patient. Other quantitative methods (mild, moderate, severe) and the visual analog scale are more difficult for the patient to understand, but they may be more useful for in-depth analysis of hearing results. In any case, further refinement of the criteria and magnitude of subjective hearing benefit is necessary if it is to be used as a routine reporting parameter.

In conclusion, there is considerable discrepancy between the pure tone audiogram results and the subjective sensation of hearing benefit. Objective pure tone audiogram parameters, including AB gap closure and AC threshold shift, are not adequate in reflecting the hearing benefit of tympanoplasty for chronic suppurative otitis media. A combination of parameters, including AB gap, AC threshold, and subjective hearing benefit score, is recommended in reporting results of tympanoplasty and in preoperative counseling of patients. Further refinement of the quantitative methods of measuring subjective hearing benefit is, however, necessary.

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ENDOLARYNGEAL MICROSURGERY AT THE ANTERIOR GLOTTAL COMMISSURE: CONTROVERSIES AND OBSERVATIONS

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There are a number of tenets regarding endolaryngeal microsurgical management of disease that involves and/or encroaches upon the anterior glottal commissure (AGC). They include avoidance of 1) bilateral epithelial incisions near the AGC, 2) removal of papillomatosis in the AGC, and 3) resection of bilateral keratosis with atypia or carcinoma at the AGC. During the last 6 years, 115 patients underwent microsurgical management of disease at the AGC: carcinoma in 20 (T1 in 15 and T2 in 5), keratosis in 41, papillomatosis in 20, and polypoid corditis (Reinke's edema) in 34. No patients with polypoid corditis developed a synechia or web. All cancers were successfully resected en bloc; 1 of the 20 patients developed a microscopic local failure that was successfully re-resected endoscopically. Eleven of the 20 cancers required excision of part of the supraglottis to establish adequate exposure for the glottic cancer resection. Eight of 15 patients with bilateral keratosis underwent staged resections. Fourteen of 15 patients with bilateral papillomatosis required staged resections. Twelve of the total 115 patients presented with a web secondary to prior microsurgery, and 3 developed a new, clinically insignificant web. The complications of management of disease in or near the AGC described by other authors were not noted in this series. This success was primarily the result of improved exposure in the AGC, which was achieved by use of larger and better-designed laryngoscopes and by resection of supraglottic tissue as necessary. Positioning these prototype laryngoscopes was facilitated by the use of elevated-vector suspension and external counterpressure.

KEY WORDS — anterior commissure, atypia, cancer, dysplasia, glottis, laryngoscopy, larynx, microlaryngoscopy, papilloma, phonosurgery, Reinke's edema, vocal cord, vocal fold.

INTRODUCTION

Endolaryngeal microsurgical management of disease at the anterior glottal commissure (AGC) has often been avoided and discouraged. Resection of cancer located at or near AGC is considered to be problematic because of inadequacy of resection, recurrence of cancer, postoperative webbing and synechiae, and dysphonia. Excision of benign lesions at the AGC has been associated with the aforementioned forms of scarring and associated hoarseness. Poor laryngoscopic exposure of the AGC could potentially be the primary or associated cause of all of these undesirable postsurgical outcomes.

Glottic Cancer. Laryngoscopic surgical management of cancer at the AGC has been controversial because of inconsistent anatomic definition of this region. The boundaries of the AGC have been ill-defined and included various amounts of the infrapetiole supraglottis, as well as an arbitrary area below the free edge of the vocal folds.[1-5]

A preponderance of the literature related to surgery at the anterior commissure has focused on the biological behavior of glottic cancer and its management. Contradictory evidence has been reported regarding the predisposition of cancer in the AGC to invade the thyroid cartilage. The contraindications to endoscopic resection are related to the differing definitions of the AGC.

If the definition of the anterior commissure is restricted to the level of the vocal folds, the anterior commissure tendon is a confluence of the vocal ligaments, inner thyroid perichondrium, and thyroepiglottic ligament.[2] Bagatella and Bignardi[6] have referred to this region as the "X" zone, and it is considered a dense, effective tumor barrier. The connective tissue diminishes rapidly above and below the anterior commissure tendon, and its sparsity permits thyroid cartilage invasion when surface disease extends in these directions from the anterior commissure tendon. Kirchner and Carter[7,8] concluded that T1a and T1b carcinomas rarely penetrate the anterior commissure tendon to invade the thyroid cartilage. Anterior commissure tumors that demonstrate cartilage invasion typically display surface invasion of the infrapetiole region[7,8] or extension to the subglottis[9] (T2 by surface stage). Sessions et al[5] also recognized that the cartilage invasion associated with surface spread above and below the insertion of the vocal folds was a reflection of greater tumor volume, rather than a predisposition of soft tissue disease at the AGC to invade cartilage.

Treatment algorithms for glottic carcinoma involving the AGC have been controversial, because of these

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uncertain patterns of spread and invasion. Further, there is often difficulty in fully exposing the anterior commissure by means of conventional laryngoscopic instrumentation.[10,11] Incomplete exposure of the anterior commissure predisposes to incorrect staging and treatment planning. Many years ago, Jackson et al[12,13] discussed the difficulties in glottal exposure, and this issue has recently been revisited.[14-18]

Zeitels[19] has received a US patent for a universal modular glottiscope, which is designed to conform to the AGC while providing optimal binocular exposure at multiple magnifications. It comprises a plurality of different-sized examining tubes that have a detachable base plate and bilateral proximal slots for angulating hand instruments. Wide exposure allows one to choose laser or cold instruments[20,21] and to safely narrow cancer-free margins.[16,22]

Endoscopic resection of early glottic carcinoma at the anterior commissure has been discouraged since Lynch[23] first described the approach in the early 20th century. Inadequate exposure and tangential visualization have often resulted in incomplete resection and a high recurrence rate.[10,24-26] These authors frequently vaporized the cancer or removed it in a piecemeal fashion. Koufman[27] and Davis et al[28] established that cancer at the anterior commissure could be resected effectively; however, it was technically difficult and dependent on exposure.[28] A number of European authors have since demonstrated the efficacy of this approach.[29-32] Adequate resection of cancer at the anterior commissure often requires resection of part of the supraglottis.[11,33]

Benign Disease. Surgeons have been reluctant to operate at the anterior commissure for benign disease, as well. It is taught that injudicious phonomicrosurgery at the anterior commissure can result in web formation, synechiae, and dysphonia.[34,35] The published literature is sparse in reports of formal analysis of these outcomes. Avoidance of simultaneous bilateral epithelial incisions at the AGC has been recommended to prevent these complications. Therefore, most surgeons do not excise papilloma from the anterior commissure, and many do not remove bilateral Reinke's edema (polypoid corditis).

The developments in phonomicrosurgery during the last decade have elucidated a number of observations and experiences that led us to question current surgical precepts regarding endolaryngeal surgery at the AGC. It seemed that the cause of the poor outcomes and complications, which were commonly described, related primarily to compromised endoscopic exposure. This prompted the present assessment of our experience with endolaryngeal microsurgery at the AGC.

MATERIALS AND METHODS

A retrospective review was performed of 115 patients who underwent microsurgical resection of a lesion(s) located at or near the AGC during the last 7 years. All surgery was performed by the senior author (S.M.Z.) between 1992 and 1998. Patients were included in the study if either a photograph and/or a map drawn at the time of the operative procedure demonstrated that the lesion(s) required an incision(s) to within 3 mm of the AGC. Patients were divided into groups according to their disease: polypoid corditis, papillomatosis, keratosis with atypia, and carcinoma.

The endolaryngeal procedure was initiated by first introducing the largest tubular glottiscope that could be placed from the lips to the vocal folds. This was typically a prototype Vaughan scope (R. Wolf Medical Instruments Corp, Vernon Hills, Ill), and more recently, a prototype universal modular glottiscope (Endocraft® LLC, Providence, RI).[19] Both of these laryngoscopes have a distal triangular lumen to accommodate to the contour of the AGC. The universal modular glottiscope has a unique linear conformation with lateral slots for angulation of hand instrumentation. The examination tubes vary in size and contour, and attach to a universal handle that can be joined to suspension apparatuses as well as different fulcrum-torsion laryngoscope holders.

The patient was placed in the Jackson position and suspended from the Boston University gallows[36] (Pilling Company, Fort Washington, Pa) to achieve elevated vector suspension. External counterpressure was applied with silk tape[15] and a laryngeal cushion.[37] The details of the technique have been previously described.[15,16,18,38] The procedures were performed primarily under 7× or 10× magnification, which is typically the highest magnification on the surgical microscope. In all benign cases, the lesion(s) was excised with cold instrumentation. Staged resections were done if a 3-mm bridge of normal epithelium could not be retained at the inner medial aspect of the AGC. A CO2 laser[21] was used to resect a number of the larger and more invasive cancers.

A subepithelial infusion of 1/10,000 epinephrine in saline[16,39,40] was performed when it was beneficial to increase the volume of the superficial lamina propria and constrict its microvasculature. Similarly, a microflap dissection technique was performed to resect pathological epithelium[16,20,22,39,41] or to preserve epithelium if the lesion (ie, cyst or polyp) was within the superficial lamina propria.[39,40,42] To obtain adequate exposure of the AGC in selected cases of keratosis with atypia, carcinoma, or papillomatosis, resection of a portion of the vestibular folds[33]

TABLE 1. DISTRIBUTION OF DISORDERS

Diagnosis	*Male*	*Female*	*Total*	*Vestibular Fold Resection*	*Infrapetiole Resection*
Cancer	16	4	20	3	8
Keratosis	28	13	41	1	2
Reinke's edema	5	29	34	0	0
Papillomatosis (all adult)	11	9	20	2	0
Total	60	55	115	6	10

and/or the infrapetiole region of the supraglottis[11] was performed with the CO_2 laser. The tissue resection was done with a repeated pulse mode; the interval and duration of the pulses varied between 0.05 and 0.1 second, the spot size was primarily 0.3 mm, and the power varied from 2 to 5 W.

The narrow-margin graded-resection approach has been previously described for resecting glottic cancer.[16,20,22] The magnification provided by the surgical microscope is critical for the success of this technique. Subsequent phonosurgical reconstruction of neocord defects is done by means of fat injection medialization and/or transcervical medialization laryngoplasty.[43] GORE-TEX is typically used to externally medialize these neocords.[44,45]

At the time of resection, photodocumentation was obtained and/or a map was drawn indicating the size, shape, location, and depth of the lesion(s). Most patients were discharged to home on the day of the procedure. All patients were instructed to abstain from voice use for the first 10 days after surgery. At approximately 10 days, the patients were examined, and most were allowed to begin gradual voice use under the direction of a speech therapist. Most patients were treated for reflux with behavior and diet counseling, the administration of proton pump inhibitors, and occasionally, prokinetic agents.

RESULTS

The distribution of disorders is delineated in Table 1. All disease in the 115 cases was adequately exposed for complete phonomicrosurgical resection, including all cancers. Eleven of the 20 glottic cancers required partial supraglottic resection for adequate exposure of the cancer and, in the majority, an extensive infrapetiole exploration. None of these cancer patients underwent postoperative irradiation, and 1 sustained a microscopic local failure 7 months after treatment. This individual, who had bilateral T1a lesions, developed a 4-mm microinvasive recurrence, which was resected endoscopically again without substantive alteration in voice quality. The mean follow-up of the cancer patients is 33 months; however, only 8 of 20 patients have more than 3 years of follow-up. There were 4 other patients with early glottic cancer who were treated with radiotherapy after a small biopsy because it was deemed they would achieve a better vocal result (see Discussion) or because of patient preference. The exposure of these individuals at the time of biopsy was such that all of them could have been technically resected.

All 3 of the cancer patients who presented with pretreatment webs (Table 2) had undergone prior endolaryngeal microsurgery. One of the 3 had a 5-mm web (Fig 1) that involved about 40% of the contralateral musculomembranous vocal fold and provided a pathway for the cancer to become a T2b lesion (Table 2). This patient, as well as another patient with a T2b lesion, underwent a complete resection of 1 side of the anterior commissure tendon and the medial aspect of the contralateral anterior insertion of the vocal ligament. The resection effectively reduced the web from 5 mm to 2.5 mm.

Prior web formation was seen on presentation in 12 of the 115 patients; 9 had benign lesions. All of these individuals had also undergone previous endolaryngeal microsurgery, and the majority had recurrent papillomatosis of the glottis. None of these individuals developed worsening of the webs that were

TABLE 2. SURGICAL PROCEDURES

Diagnosis	*Unilateral Resection*	*Bilateral Resection*	*Bilateral Staged Resection*	*Prior Web*	*New Web*
Cancer (n = 20)	14 T1a 3 T2a	1 T1b 2 T2b	0	3*	0
Keratosis (n = 41)	26	7	8	3	1
Reinke's edema (n = 34)	0	34	0	0	0
Papillomatosis (n = 20)	5	1	14	6	2
Total (n = 15)	41	51	23	12	3

*Web decreased from 5.5 mm to 2.5 mm in length in 1 patient.

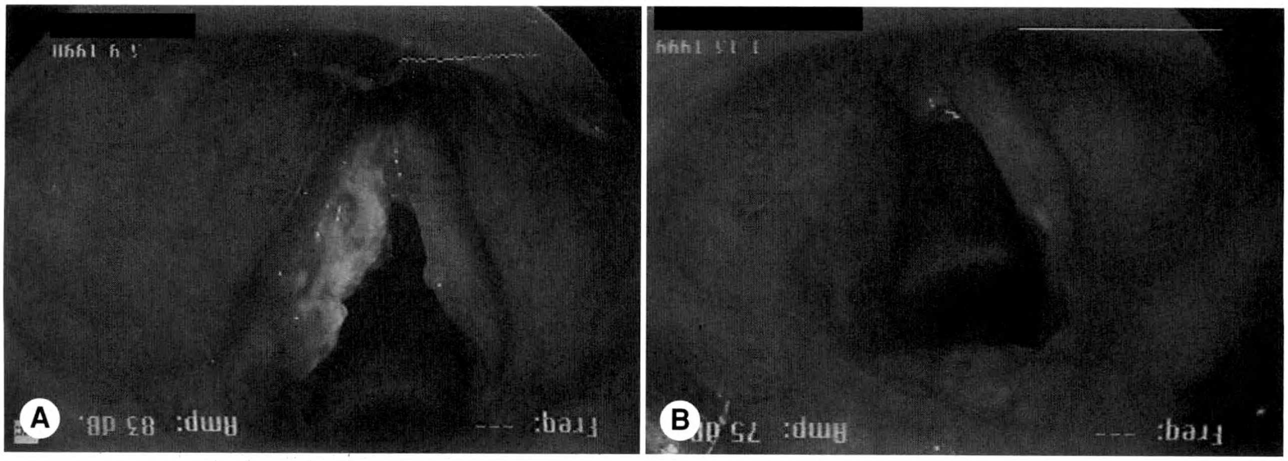
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Fig 1. Patient with T2b carcinoma of left vocal fold. A) Pretreatment. Web served as pathway for disease to invade contralateral vocal fold. Cancer was more pronounced on undersurface of web. B) Subsequent to endoscopic resection of vestibular fold, infrapetiole region, and glottic carcinoma, neocord was augmented with fat. Residual web that is seen is significantly reduced in size and is below free edge of vocal fold.

present. Three patients developed new webs, all of which were under 2.5 mm in length, and none created significant airway or vocal dysfunction. The 2 papilloma patients underwent staged resections for disease that was encompassing the anterior commissure and both anterior vocal folds. The patient with keratosis had a similar geographic presentation anteriorly. He is schizophrenic and has significant cardiac problems, so it was elected to resect the disease during 1 general anesthesia and to accept inevitable web formation. Remarkably, he had no subjective alteration in his vocal quality because of underlying Reinke's edema.

DISCUSSION

As direct laryngoscopic surgery[46] evolved through this century,[47] a paradox developed in the management of disease at the AGC. Despite the fact that narrow-lumen monocular tubular glottiscopes[48] could be passed to the vocal folds with relative facility in most patients with local anesthesia, these scopes were ill-suited for precise instrumental manipulation of the glottal tissue. The early direct-laryngoscopic procedural interventions consisted mainly of single-handed tissue avulsion. As the diameter of the glottiscopes increased, tissue handling improved, but exposure of the anterior commissure deteriorated. This problem could be only partially remedied by optimizing patient position[14,16-18,49] and the application of external counterpressure.[15,18,37] In a recent study, it was demonstrated that wider-lumen glottiscopes frequently require significantly greater force to expose the AGC and that in many patients, a large laryngoscope cannot expose the AGC at all.[17]

The era of microlaryngoscopy precipitated the need for an ideal glottiscope shape, contour, and size for laryngoscopic exposure and tissue handling of the vocal folds. The ideal glottiscope would retain a balance of characteristics. It should have a wide-lumen examining tube that can accommodate the oculars of the surgical microscope for stereoscopic visualization at most magnifications. The tube should allow for bimanual tissue manipulation and have an outer diameter that can be passed through the oral cavity and pharynx to the anterior commissure. It is hoped that the universal modular glottiscope system[19] will satisfy these goals for a majority of surgeons.

During the ongoing development of direct laryngoscopic surgery for disease at the AGC, a number of traditions and precepts evolved based on poor clinical outcomes. Our observations suggested that 1) many poor technical results could be remedied by improved exposure of the AGC, as well as by carefully placing and/or staging the incisions, and 2) endoscopic control of disease at the AGC had not been a problem.

This study used 2 main parameters to assess laryngoscopic management of disease at the AGC: 1) successful removal of the disease, and 2) development of postsurgical anterior commissure webbing and synechia. Cancer, atypia, papilloma, and polypoid corditis (Reinke's edema) were chosen as disorders for study because of the frequency with which they extend to the AGC.

Complete excision of a diseased tissue is the fundamental requirement for the treatment of vocal fold cancer at the anterior commissure. The literature is replete with admonitions about the inability to consistently eradicate cancer from the AGC by laryngoscopic methods.[10,23-25] However, this problem was not encountered in this series, in which all vocal fold cancers were resected with clear margins (Fig 2). The sole patient with a local recurrence had a microinva-

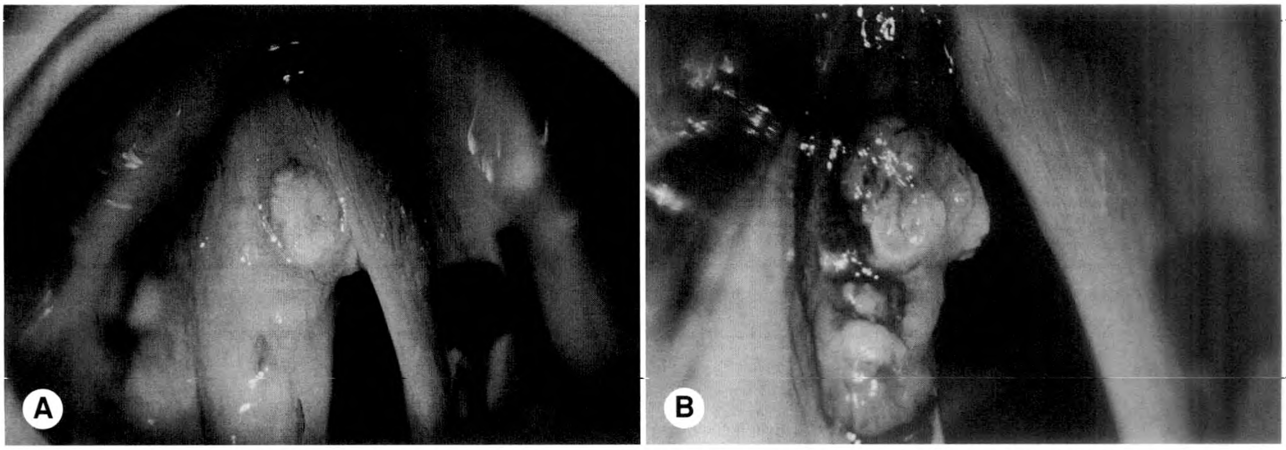

Fig 2. Patient with T1a carcinoma. A) Carcinoma is seen at anterior commissure subsequent to subepithelial infusion of saline with 1/10,000 epinephrine. B) Cancer has been outlined and dissected as inferiorly based microflap. It has been laid back in place to display resection margin extending into anterior commissure.

sive lesion that was re-resected endoscopically, and he has had no subjective voice deterioration because of long-term underlying Reinke's edema. In the patients in whom irradiation was administered for a better vocal outcome, effective cancer resection would have required resection of the entire anterior commissure tendon. Resection of 1 vocal fold in combination with dissociation of the second vocal fold from the anterior thyroid lamina typically results in a poor voice, because of the severe structural disturbance of the glottal soft tissues.

Although endoscopic control of cancer has been excellent, definitive statements about oncological validity will require further examination when the majority of patients have more than 3 years of follow-up. The effectiveness of phonomicrosurgical management of cancer in the AGC is unquestionably the result of improved exposure, which is essential for any surgical oncological procedure.

The exposure was optimized in 4 ways. First, the glottiscopes used in this series retained a triangular configuration[13,14,19,50] to conform to the AGC and to distract the supraglottal tissues out of the visual surgical field. Second, elevated vector suspension[16] was used in all patients, which is a prerequisite for placement of the widest possible glottiscope.[15,16,18] This can only be achieved with a true suspension gallows,[15,18,36] not a chest-support torsion-fulcrum laryngoscope holder, as is used by most surgeons. Third, external counterpressure[15,18,37] on the lower laryngeal framework was routinely used, as has been suggested since the turn of the century.[51]

Finally, laser resection of uninvolved supraglottic tissue (infrapetiole region and anterior vestibular folds) was done in any case in which the perimeter of the lesion could not be visualized in its entirety.[11,33] This allowed for positioning of the triangular glottiscope adjacent to the inner thyroid lamina above the AGC, which facilitated clear resection of part or all of the anterior commissure tendon. Complete resec-

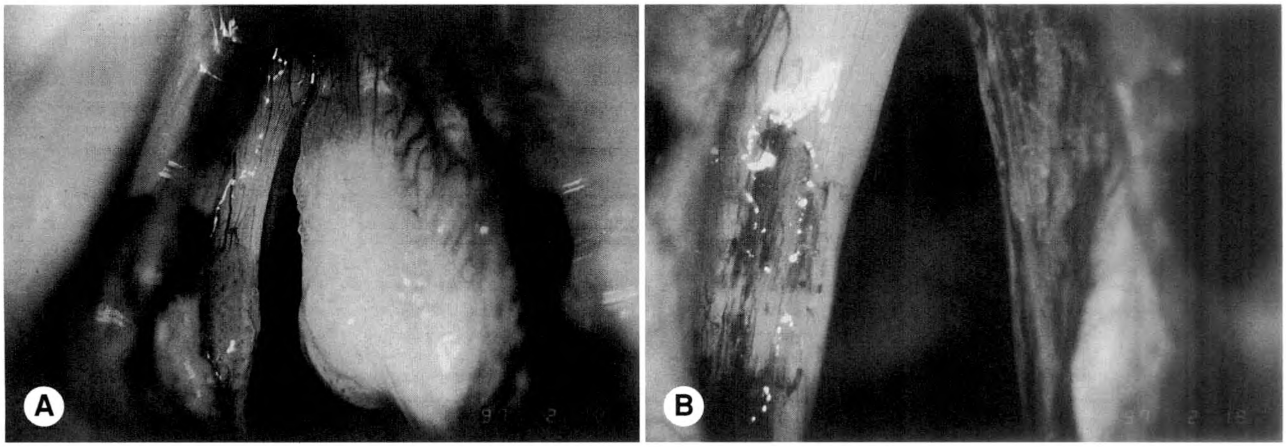

Fig 3. Patient with papillomatosis. A) Diffuse papillomatosis is seen extending to anterior commissure on right vocal fold and smaller focus on left. Subepithelial infusion has been done with 1/10,000 saline and epinephrine. B) This is sole patient with papillomatosis who underwent resection of bilateral disease in 1 procedure. Residual normal superficial lamina propria is well seen.

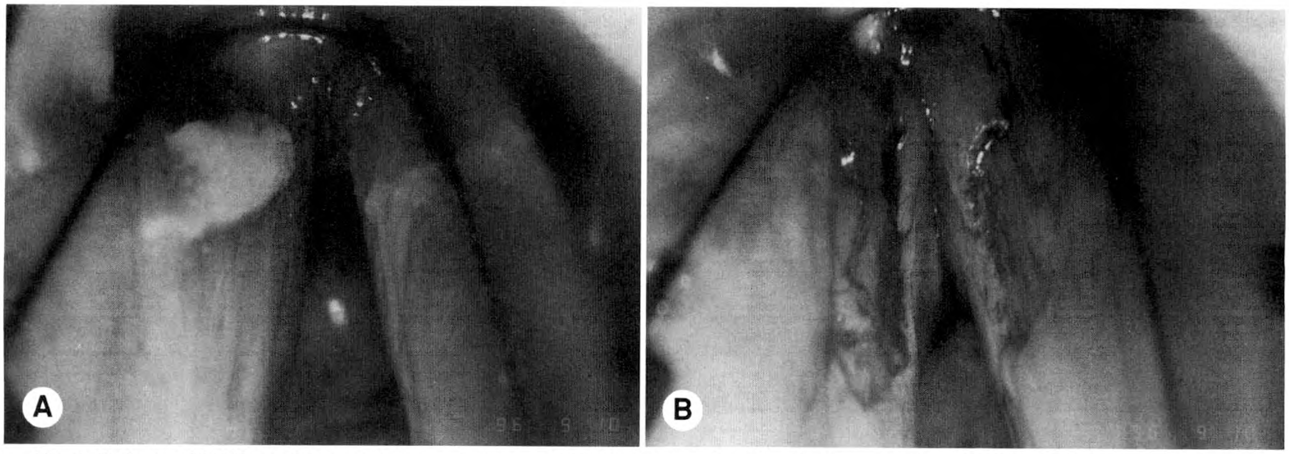

Fig 4. Patient with bilateral keratosis on superior surface of both vocal folds. A) Pretreatment. B) Both lesions were resected in 1 procedure. Note that inner aspect of anterior commissure is intact.

tion of the vocal fold cancer necessitated removal of 1 vestibular fold in 3 of the 20 cancer patients, and the infrapetiole region with a vestibular fold(s) as well in 8 of the 20 patients.

All 20 patients with glottal papillomatosis and 41 patients with keratosis with atypia underwent complete resection of their disease. One of the 15 papilloma patients with bilateral disease (Fig 3) and 7 of the 15 keratosis patients with bilateral disease (Fig 4) underwent resection on both sides in a single procedure. Complete exposure and resection of the disease in the atypia population is critical for obtaining an accurate diagnosis and for treatment.[16] Complete microflap resection of papillomatosis at the anterior commissure facilitated long-term control of most patients in whom it was their initial presentation, and of many patients with recurrent disease.[41]

The development of webs and synechia was not a significant problem in this series. Twelve patients presented with small AGC webs from prior microsurgery, and none of these individuals developed a substantial change of the web. One patient presented with a 5-mm web and a T2b glottic cancer, and endoscopic laser resection not only treated the neoplastic process, but also reduced the length of the web to 2.5 mm.

Bilateral incisions were made if a 3-mm area of uninvolved epithelium could be preserved in the internal aspect of the AGC (Figs 3B and 4B), and staged procedures were done if the disease extended across the AGC. Three of the 23 patients who underwent staged resection of disease (papilloma, atypia) in the anterior commissure and on both vocal folds developed new, minor webbing that did not affect the airway or voice (subjective assessment). No patients who underwent bilateral incisions or resection of unilateral disease developed a web or a synechia.

Although small (<2.5 mm) acquired webs may result in an alteration of fundamental frequency or limitations in maximal range tasks (pitch or frequency and loudness or intensity), these webs probably do not cause severe dysphonia, hoarseness, jitter, shimmer, or an alteration in the signal-to-noise ratio. In larger webs, there is a fixed, foreshortened glottal aperture. We believe that the primary cause of vocal perturbation is the disordered passage of the subglottal airstream through the vocal fold tissue not involved with the web. This is confirmed by the stroboscopic examination, which typically reveals that the impaired vocal fold vibration and associated dysphonia is secondary to associated stiffness of the epithelium of the musculomembranous vocal folds, which is not associated with the web (Zeitels, unpublished data). The stiffness of the epithelium and the web are different visual manifestations of the diffuse, traumatically induced fibrosis. Endoscopic or open surgical lengthening of the vocal folds will not substantially improve the voice if the underlying superficial lamina propria is not reconstructed. Unfortunately, an easy, effective procedure for superficial lamina propria augmentation is not yet available.

CONCLUSIONS

1. The key to successful phonomicrosurgery at the AGC is wide exposure. This requires larger and better-designed laryngoscopes, elevated vector suspension, and external counterpressure.

2. Glottic cancer at the AGC can be technically excised with clear margins by endolaryngeal microlaryngoscopic resection.

3. Effective microsurgical resection of early glottic cancer at the AGC often requires excision of the vestibular folds and/or the infrapetiole region of the supraglottis to provide adequate exposure and visualization.

4. The oncological safety of microsurgical treat-

ment of cancer at the AGC will require re-examination of this cohort of patients when the majority of individuals have been followed for more than 3 years.

5. Bilateral glottal disease can be managed in 1 procedure if 3 mm of normal epithelium can be maintained at the internal medial aspect of the AGC.

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MONTGOMERY® THYROPLASTY IMPLANT FOR VOCAL FOLD IMMOBILITY: PHONATORY OUTCOMES

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Forty-three patients with a diagnosis of unilateral vocal fold immobility underwent thyroplasty type I with the Montgomery® Thyroplasty Implant System. Preoperative and postoperative evaluations were completed by means of videostroboscopic, acoustic, and aerodynamic measures. Clinicians' perceptions of vocal quality and patients' satisfaction with the surgery and vocal quality were determined. Improvements after surgery were observed for glottal closure, vocal fold amplitude, mucosal wave activity, average intensity, maximum intensity range, maximum phonation time, glottal airflow, average sound pressure, and subglottal pressure. Average postsurgical fundamental frequency values fell within normal limits and did not display significant changes relative to presurgical values. The clinicians' perceptual evaluations indicated an improvement in voice quality for most patients. A majority of patients expressed satisfaction with the surgery and resulting voice quality. The results of the present study, in combination with the surgical advantages that have been described for the Montgomery® Thyroplasty Implant System, support the view that this approach offers an attractive alternative for treating unilateral vocal fold immobility.

KEY WORDS — laryngoplasty, Montgomery® Thyroplasty Implant System, thyroplasty, unilateral vocal fold paralysis, vocal fold immobility, vocal fold medialization, voice.

INTRODUCTION

Thyroplasty type I has become the procedure of choice for patients with dysphonia caused by vocal fold immobility.[1-6] This laryngeal framework surgery results in medial displacement of the immobile vocal fold by placing an implant between the thyroid cartilage and the inner thyroid perichondrium through a window created in the thyroid cartilage.

Isshiki et al[2] initially reported using, as the implant, a cartilage wedge taken from the thyroid cartilage contralateral to the immobile vocal fold. Later, implants made from synthetic materials such as silicone, were used.[4,6,7] The hand-carved silicone implants have been an improvement over cartilage because of the relative ease in shaping the material to achieve an appropriate shape, size, and position. However, silicone is difficult to carve, and the edges can become rough or irregular.[8] Other disadvantages to the hand-carved silicone implants include 1) possible migration superiorly, inferiorly, medially, or laterally; 2) possible rotation that causes them to present obliquely in the glottis; and 3) an increase in the length of surgery.[8]

The Montgomery® Thyroplasty Implant System[5,8] (Boston Medical Products, Inc, Westborough, Mass) consists of a medialization implant, measuring devices, and surgical instruments. The new implant is constructed from a biocompatible, medical-grade polymer and is triangular in shape. The Montgomery® Thyroplasty Implant offers several advantages over a custom-carved silicone implant: 1) it is readily available, eliminating the need to either fashion or alter the implant at the time of surgery (5 sizes for the male larynx; 5 sizes for the female larynx); 2) with a measuring device, the precise size can be easily determined during the procedure by visualizing the larynx with a fiberoptic laryngoscope and listening to the patient's phonatory function; 3) the implant is easily inserted and snapped into place, and no sutures are necessary; 4) once it is inserted, the chance for medial or lateral displacement is remote, so complications are decreased; 5) if indicated, the implant can be easily removed without damage to either the cartilaginous or soft tissue structures; 6) because of the uniformity of the base design, different-sized implants can be inserted with no change in the thyroid lamina window; and 7) the triangular shape of the implant, with its greatest dimension posteriorly, is designed to assist with posterior glottal gap closure. Thus, there are apparent surgical advantages with the new implant system; however, phonatory out-

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TABLE 1. EVENTS ASSOCIATED WITH UNILATERAL VOCAL FOLD IMMOBILITY IN STUDY GROUP

Events	*No. of Cases*
Thoracic surgery	8
Base of skull tumor surgery	8
Thyroidectomy	6
Viral	4
Intubation injury	3
Carotid endarterectomy	3
Carotid artery angioplasty	1
Cancer of esophagus	2
Anterior cervical disk surgery	1
Resection of hypopharyngeal diverticulum	1
Cricopharyngeal myotomy	1
Idiopathic	5
Total	43

comes have not been reported.

Several investigators have reported phonatory outcomes of thyroplasty with other medialization implants.[9-21] Measures used to determine vocal improvement have included clinician and/or patient perceptions,[9,10,13,14,16,19,20] perceptual judgments of videolaryngoscopy,[10,12,14,16,18,19,21] and acoustic and/or aerodynamic assessments.[9-17,19] In general, data have demonstrated improvement in phonatory function after thyroplasty.

The purpose of the present study was to evaluate phonatory outcomes in patients treated for unilateral vocal fold immobility with the Montgomery® Thyroplasty Implant.

MATERIALS AND METHODS

Forty-three patients (20 women, age range from 19 to 83 years, mean age of 55; 23 men, age range from 15 to 81 years, mean age of 63) served as subjects. Assessments were performed for each patient before and after thyroplasty implant surgery (minimum of 6 weeks after surgery; range, 6 weeks to 2 years 6 months). Each patient had received a diagnosis of unilateral vocal fold immobility (16 right; 27 left). A summary of the events associated with the onset of unilateral vocal fold immobility in the study group is shown in Table 1. Seven patients had documented hearing losses. An additional 15 patients failed the hearing screening at the time of the preoperative evaluation (screened at 20 dB hearing level at 500, 1,000, 2,000, and 4,000 Hz, bilaterally, in a sound-treated room). One patient had undergone laryngeal surgery for bilateral polyps in 1977, and another patient had undergone cricopharyngeal myotomy 1 month before the preoperative evaluation. No patients underwent voice therapy between the preoperative and postoperative evaluations. Data were collected from March 1993 to November 1996.

SURGICAL TECHNIQUE

Each patient underwent thyroplasty type I surgery with the Montgomery® Thyroplasty Implant System. All operations were performed by the second author. The thyroplasty system and surgical procedures have been described previously.[5,8]

PERCEPTUAL JUDGMENTS OF VOCAL QUALITY

Judgments were made by 3 speech-language pathologists experienced in the evaluation and treatment of patients with voice disorders. The stimuli were digitized samples of sustained /a/ vowels (1 preimplant and 1 postimplant) from 33 of the 43 patients. The methods used to acquire the vowel samples are described below (see Acoustic Assessment). The stimuli were presented in pairs to each of the judges over headphones by means of the digital-to-analog output and playback utilities of the computer-based system used for acoustic analysis. For each stimulus pair, judges indicated which of the 2 samples had the most disordered vocal quality. If both samples of a stimulus pair were perceived to have the same quality, judges indicated whether the samples were normal or disordered (mild, moderate, or severe). The order of stimulus presentation was randomized, and judges were blinded as to whether samples were from preimplant or postimplant recordings. Each judge repeated the evaluation of a different random ordering of all stimulus pairs on a separate day.

PATIENTS' SATISFACTION WITH SURGERY AND VOCAL QUALITY

Surveys were sent to each of the patients following postimplant evaluations. The patients were asked to indicate how they felt about the quality of their voices at that time, and how satisfied they were with the surgery. The responses were used to determine patient satisfaction.

VIDEOENDOSCOPY WITH STROBOSCOPY

Videostroboscopic recordings were obtained before and after implantation for each patient by means of transnasal flexible (Olympus ENF-P-3) and/or transoral rigid (Kay Elemetrics 70) laryngeal endoscopy. (Both types of examinations were attempted on all patients.) The video recordings were presented to a group of experienced speech-language pathologists and otolaryngologists at weekly case review meetings (voice rounds), and ratings of glottal closure, amplitude of vocal fold vibration, and mucosal wave activity were arrived at via group discussion and consensus.

ACOUSTIC ASSESSMENT

Digital audiotape (DAT) recordings of patients'

TABLE 2. JUDGMENTS OF VIDEOSTROBOSCOPY

	Preimplant			*Postimplant*		
	N	*CNR*	*A*	*N*	*CNR*	*A*
Glottal closure	2	4	37	34	0	9
VF amplitude of IVF	13	15	15	29	3	11
VF amplitude of MVF	19	15	9	36	2	5
Mucosal wave of IVF	13	14	16	31	3	9
Mucosal wave of MVF	22	10	11	37	2	4

N — normal; CNR — could not rate; A — abnormal; VF — vocal fold; IVF — immobile vocal fold (16 right, 27 left); MVF — mobile vocal fold (27 right, 16 left).

voices were made with a Tascam DA-30 Digital Audio Tape Deck in a sound-treated room. An electret condenser microphone was held a constant distance of 15 cm from the lips with a headband suspension device. The output of the microphone was fed to a preamplifier and then recorded on DAT. A calibration tone of known intensity (in decibels sound pressure level [SPL]) was recorded before each patient evaluation and was used as a reference for acoustic analyses. Recordings were made as each patient performed a series of standard tasks: reading a standard passage (Rainbow Passage), sustaining the vowel /a/ as long as possible on 1 breath, and sustaining /a/ at the softest and, then, the loudest intensities possible. The DAT recordings were digitized with the Computerized Speech Lab (CSL; model 4300/4300B, Kay Elemetrics) interfaced with a DTK 486s computer. Analyses of average fundamental frequency (hertz) and intensity for reading (decibels SPL), maximum phonation time (seconds), and maximum intensity range (decibels SPL) were completed with CSL software application programs.

AERODYNAMIC ASSESSMENT

Estimates of subglottal air pressure, glottal airflow, and sound pressure were obtained noninvasively from repetitions of /pae/ syllable strings with an Aerophone (Kay Elemetrics) system interfaced with a DTK 486s computer. Patients were instructed to repeat /pae/ at a comfortable level while the following measures were obtained: 1) oral airflow measured with a pneumotachograph mounted in a face mask, 2) intraoral air pressure measured with a translabially placed catheter connected to a pressure transducer, and 3) sound pressure measured with an electret microphone affixed to the flow pneumotachograph at approximately 15 cm from the patient's lips. Recordings were made in a sound-treated room. The flow and pressure signals were digitized and analyzed with the Aerophone II-1992 software application program. Indirect estimates of average glottal airflow rate (in liters per second), subglottal air pressure (in centimeters of water), and sound pressure values (in decibels SPL) were derived from the analyses.

STATISTICAL ANALYSIS

We completed *t*-tests for paired samples using the SYSTAT 7.0 program to compare the significance of the preimplant versus postimplant data by sex for each of the acoustic and aerodynamic measures.

RESULTS

PERCEPTUAL JUDGMENTS OF VOICE QUALITY

Voice quality was considered to have improved after surgery if the preimplant voice sample was judged to be more disordered than the postimplant sample. An overall average of 80% of the patients were judged to exhibit an improvement in postimplant voice quality (27/33 or 82% on the first day; 26/33 or 79% on the second day). With the exception of 1 patient rated on the second day, the same 5 patients were judged to not demonstrate consistent improvement in voice quality for both rating sessions. The average interjudge reliability was 87%. The intrajudge reliability values for the 3 judges were 100%, 82%, and 79%.

PATIENTS' SATISFACTION WITH SURGERY AND VOICE QUALITY

Thirty-nine of the 43 patients responded to the mailed survey. Of the 39 patients, 100% responded that they would be willing to repeat the surgery if necessary, 95% expressed positive feelings related to their satisfaction with the results of their surgery, and 80% chose responses indicating positive feelings regarding satisfaction with voice quality.

VIDEOENDOSCOPY WITH STROBOSCOPY

The videoendoscopic parameters evaluated were 1) glottal closure, 2) amplitude of vocal fold vibration, and 3) mucosal wave activity (Table 2). It should be noted that for each of the parameters evaluated, some of the patients' data could not be evaluated because of compression of supraglottal structures secondary to vocal straining. Improvement could not be evaluated in cases in which compression of supraglottal structures precluded preimplant assessments.

Glottal Closure. Glottal closure was judged to be normal if the glottis closed completely or if there was a posterior chink of the cartilaginous glottis only. Before surgery, only 2 patients displayed normal glottal closure, 4 could not be evaluated, and 37 had abnormal closure. After surgery, 34 patients displayed normal glottal closure, all patients could be evaluated, and only 9 had abnormal closure. Of the 9 patients with abnormal postimplant closure, 6 showed an improvement in closure compared to their preimplant status, and 1 could not be judged with respect to improvement because preimplant glottal closure

TABLE 3. ACOUSTIC RESULTS

	Preimplant	*Postimplant*
Average Fo (Hz)		
Male (N = 12)	139	123
Female (N = 18)	202	185
Average intensity (dB SPL)		
Male (N = 19)	64	68*
Female (N = 19)	64	67*
Maximum intensity range (dB SPL)		
Male (N = 16)	34	45*
Female (N = 19)	35	42*
Maximum phonation time (s)		
Male (N = 18)	8.3	19.3*
Female (N = 19)	6.7	12.3*

Fo — fundamental frequency; SPL — sound pressure level.
*$p < .05$.

could not be rated.

Vocal Fold Amplitude. The vocal fold amplitude was rated for the immobile vocal fold (IVF) and the mobile vocal fold (MVF) separately as normal (greater than normal or normal) or abnormal (slight decrease, moderate decrease, severe decrease, or absent) for each vocal fold per patient. Of the immobile vocal folds, 27 were left, and 16 were right.

For the IVF, before the implant procedure, 13 patients displayed normal amplitude of vocal fold vibration, 15 could not be evaluated, and 15 had abnormal vibration amplitude. After the implant procedure, 29 patients displayed normal amplitude of vocal fold vibration, 3 could not be evaluated, and 11 had abnormal vibration amplitude. Of the 11 patients with abnormal postimplant vibration amplitude, 2 showed improvement, 4 did not show improvement, and improvement could not be evaluated in 5 patients.

Improvement was noted for many patients in the MVF, also. Before surgery, 19 patients displayed normal amplitude of vocal fold vibration, 15 could not be evaluated, and 9 had abnormal vibration amplitude. After surgery, 36 patients displayed normal amplitude of vocal fold vibration, 2 could not be evaluated, and 5 had abnormal vibration amplitude. Of the 5 patients with abnormal postimplant vibration amplitude, 1 showed no improvement, and improvement could not be evaluated in 4 patients.

Mucosal Wave Activity. The IVF was evaluated separately from the MVF. The mucosal wave activity was rated as normal (greater than normal or normal) or abnormal (slight decrease, moderate decrease, severe decrease, or absent).

The number of patients who displayed normal mucosal wave activity in the IVF increased from 13 (preimplant) to 31 (postimplant). Fourteen patients' data could not be evaluated, and 16 patients had abnormal mucosal waves before surgery. After surgery, 3 could not be evaluated and 9 had abnormal mucosal waves. Of the 9 patients with abnormal postimplant mucosal wave activity, 2 showed improvement, 5 did not show improvement, and improvement could not be evaluated in 2 patients.

As with vocal fold amplitude, improvement was noted in the MVF mucosal wave activity for many patients after surgery. Before the implant procedure, 22 patients displayed normal mucosal wave activity, 10 could not be evaluated, and 11 had abnormal mucosal waves. After the implant procedure, 37 patients displayed normal mucosal wave activity, 2 could not be evaluated, and 4 had abnormal mucosal waves. Of the 4 patients with abnormal postimplant mucosal wave activity, 1 showed no improvement, and improvement could not be evaluated in 3 patients.

ACOUSTIC ASSESSMENT

The acoustic parameters evaluated were 1) average fundamental frequency for reading, 2) average intensity for reading, 3) maximum intensity range, and 4) maximum phonation time (Table 3). Men and women were evaluated separately. The .05 level of significance was adopted for this study. It should be noted that there were missing values for some patients for some parameters. Cases were deleted from the statistical analyses when there were missing data. Data were missing when the voice signals were unanalyzable or when there were technical difficulties. The numbers of subjects included in each of the analyses appear in Table 3.

Average Fundamental Frequency for Reading. A comparison of preoperative versus postoperative measures indicated that the average fundamental frequency for reading decreased for the men (139 to 123 Hz) and for the women (202 to 185 Hz). Both the preimplant and postimplant values were within the normal ranges for men (86 to 156 Hz)[22] and for women (163 to 251 Hz).[23] The decrease in average fundamental frequency was not statistically significant for the men ($p = .057$) or the women ($p = .08$). It should be noted that before implantation, there were 10 patients whose voices were either too aphonic or too aperiodic to analyze. After implantation, all patient data were analyzable which demonstrated an improvement in voice. However, because these 10 patients did not have both "pre" and "post" data, they were excluded from the statistical analyses.

Average Intensity for Reading. Before implantation, the average intensity for reading for both the male and female groups was 64 dB SPL. After im-

TABLE 4. AERODYNAMIC RESULTS

	Preimplant	*Postimplant*
Average glottal airflow (L/s)		
Male (N = 19)	0.55	0.22*
Female (N = 19)	0.38	0.18*
Average estimated subglottal pressure (cm H2O)		
Male (N = 19)	10.2	9.1
Female (N = 19)	8.6	8.4
Average sound pressure (dB SPL)		
Male (N = 17)	70.0	76.9*
Female (N = 19)	72.9	75.7

*$p < .05$.

plantation, the average intensity for the male group increased significantly, to 68 dB SPL ($p = .002$), and the average intensity for the female group increased significantly, to 67 dB SPL ($p = .001$). Both of these increases brought the intensity closer to the normal means for men (70 dB SPL) and women (68 dB SPL; normative data from S. Ryan and M. P. Gelfer, unpublished observations, 1993).

Maximum Intensity Range. Before implantation, the average maximum intensity range for the men was 34 dB SPL. After implantation, the average maximum intensity range for the group increased significantly ($p = .001$), to 45 dB SPL, which was closer to the normal mean of 54.8 dB SPL.[24] The women's data also displayed a significant increase ($p = .007$), from 35 dB SPL to 42 dB SPL, which was closer to the normal mean of 51 dB SPL.[24]

Maximum Phonation Time. Statistically significant changes were seen in maximum phonation time for both the men ($p < .05$) and the women ($p < .05$). The average maximum phonation time for the men increased from 8.3 seconds to 19.3 seconds. For the women, the average maximum phonation time increased from 6.7 seconds to 12.3 seconds. Both the men's and women's postimplant values were closer to the reported clinical threshold screening values of 20 seconds for men and 15 seconds for women.[25]

AERODYNAMIC ASSESSMENT

The aerodynamic parameters evaluated were 1) average glottal airflow rate, 2) average subglottal air pressure, and 3) average sound pressure (Table 4). Men and women were evaluated separately. It should be noted that there were missing values for some patients for some parameters. Cases were deleted from the statistical analyses when there were missing data. The numbers of subjects included in each of the analyses appear in Table 4.

Average Glottal Airflow. Both groups demonstrated statistically significant decreases ($p = .001$ for men; $p < .05$ for women) in average glottal airflow. The average values decreased, from 0.55 L/s to 0.22 L/s for the men, and from 0.38 L/s to 0.18 L/s for the women. Before implantation, both the male and the female values fell above the normal range (0.05 to 0.33 L/s for men; 0.08 to 0.20 L/s for women),[26] and after implantation, they fell within the normal range.

Average Subglottal Pressure and Average Sound Pressure. The average subglottal pressure value for the men decreased from a preimplant value of 10.2 cm H2O to a postimplant value of 9.1 cm H2O. This postimplant decrease was not statistically significant ($p = .341$) for the men, but the postimplant value did fall at the high end of the normal range for men (3.5 to 9.1 cm H2O).[26] The average subglottal pressure value for the women decreased, from 8.6 ("pre") to 8.4 ("post") cm H2O. The decrease was not statistically significant ($p = .699$), and both preimplant and postimplant values exceeded the normal range (4.0 to 7.6 cm H2O).[26]

Midvowel SPL values were also obtained from the syllables produced during aerodynamic testing. The average values for the men increased, from 70.0 to 76.9 dB SPL, and those for the women, from 72.9 to 75.7 dB SPL. The increase was statistically significant for the men ($p < .05$), but not for the women ($p = .065$). Reported normative values for men (72.9 to 86.1 dB SPL)[26] and women (68.4 to 84.4 dB SPL)[26] indicate that the men's preimplant values were slightly reduced, the women's preimplant values were within the normal range, and both the male and female values were within the normal range after surgery.

To take into account the fact that SPL is highly correlated with subglottal pressure during voice production,[27] we computed ratios between the log10 of subglottal pressure and SPL, and compared preoperative versus postoperative values using *t*-tests for paired comparisons. The results indicated a statistically significant difference for male values ($p = .032$), but not for female values ($p = .196$).

DISCUSSION

The intent of the present study was to determine the effectiveness of the Montgomery® Thyroplasty Implant System with respect to phonatory outcomes in patients with unilateral vocal fold immobility. The findings from the present study were generally comparable with those reported in previous studies of phonatory outcomes after thyroplasty.

CLINICIANS' PERCEPTUAL JUDGMENTS OF VOCAL QUALITY

Past studies[9,14,16,20] have reported perceptions of vocal quality (breathiness, hoarseness, and/or straining) for preoperative and postoperative recordings of patients' voices. A marked reduction in breathiness after surgery has been indicated in most cases.[9,14,16,20] Less-clear improvement has been reported with respect to hoarseness[14,16,20] and straining.[16]

In the present study, a different approach was chosen, in which overall improvement in vocal quality was evaluated. The clinicians' perceptual judgments indicated what percentage of patients' postimplant voices sounded better than the preimplant voices. The results suggested that 80% of the patients improved with respect to voice quality.

PATIENTS' SURVEY RESPONSES

In the present study, patients' survey responses indicated high satisfaction with surgery (95%) and with vocal quality (80%). These findings were similar to those reported by Gray et al[13] from a questionnaire-based study that indicated that 92% of the patients (N = 15) approved or were highly satisfied with the surgery, and 73% were satisfied with their voices. Harries and Morrison[19] also reported good-to-excellent scores with respect to patients' satisfaction with the effect of medialization procedures on their voices.

VIDEOENDOSCOPY WITH STROBOSCOPY

Glottal Closure. In the present study, glottal closure improved significantly after medialization with the Montgomery® Thyroplasty Implant System. Several studies[14,16,18,19,21] reported similar findings after thyroplasty type I, although patient selection and/or surgical procedures varied across studies, making direct comparisons somewhat difficult. For example, in LaBlance and Maves' study,[14] all patients underwent thyroplasty type I, as in the present study. However, some of the patients in the Thompson et al[18] study underwent arytenoid adduction and/or cricopharyngeal myotomy in addition to thyroplasty type I, and in the Lu et al[16] study, some patients underwent thyroplasty type III and/or cricopharyngeal myotomy in addition to thyroplasty type I. Gorham et al[21] selected patients with glottic insufficiency for their study; however, not all patients presented with vocal fold immobility, as in this study.

Vocal Fold Amplitude. In the present study, improvement in vocal fold amplitude was noted for both the IVF and the MVF for most patients after surgery. Thompson et al[18] also studied vocal fold amplitude after thyroplasty. Thompson et al were unable to judge change in vocal fold amplitude for the paralyzed vocal fold (PVF), because the preoperative findings were too random or variable. The nonparalyzed vocal fold (NPVF) was judged to be normal after surgery for 10 of 12 patients. It should be noted that the patients included in the Thompson et al study also underwent arytenoid adductions.

Mucosal Wave Activity. One of the biggest advantages of medialization thyroplasty over other methods of treating vocal fold immobility, such as Teflon injection, is that the cover-and-body relationship that is necessary for vocal fold vibration is better preserved. In the case of vocal fold immobility, an improvement in mucosal wave activity reflects an improvement in vocal fold adduction and should affect not only the IVF, but also the MVF. In this study, improvement was noted in both the IVF and the MVF for many patients. In a similar study, Thompson et al[18] reported that 9 of 12 patients exhibited normal mucosal wave activity in the PVF (referred to as the IVF in this study) after surgery, 2 of the 12 improved, and 1 of the 12 remained the same. As regards the NPVF (referred to as the MVF in this study), Thompson et al observed that 10 of 12 were normalized after surgery.

ACOUSTIC ASSESSMENT FINDINGS

Average Fundamental Frequency. In the present study, neither the men's nor the women's average fundamental frequency for reading changed significantly after medialization thyroplasty. Both the men and women in the present study were producing average fundamental frequency values within the reported normative range both before and after surgery. A slight decrease in the group mean was noted in the present study, as might be expected if the subjects were hyperfunctioning and/or simply raising their pitch before surgery to help compensate for the immobile vocal fold. Others[14,16] analyzed recordings of sustained vowels before and after thyroplasty and reported similar findings. Sasaki et al[17] reported a significant increase in average fundamental frequency for reading after thyroplasty.

Average Intensity. The average intensity for reading increased significantly in the present study. This finding is comparable to that of Sasaki et al,[17] who also used a reading passage for the speech task. Adams et al[9] did not find a significant difference in the average intensity values of conversational speech after thyroplasty in their study. Contrary to findings from the present study, Leder and Sasaki[15] found a significant decrease when comparing 5 women's preoperative versus postoperative findings. (The time between surgery and postoperative evaluations averaged 40 months.) Leder and Sasaki stated that the decrease might have been attributable to a decrease in vocal effort with time. The differences between current and past findings might be related to the speech

TABLE 5. REPORTED AVERAGE MAXIMUM PHONATION TIMES AFTER THYROPLASTY

Authors	*Preop (s)*	*Postop (s)*
Sasaki et al[17]	5.50	9.30
LaBlance and Maves[14]	7.75	16.63
Bryant et al[12]*	3.22	12.00
Lu et al[16]	6.21	12.80
Adams et al[9]	5.60	10.90
Leder and Sasaki[15]	5.70	9.80
Harries and Morrison[19]	5.00	10.00
Present study (men)	8.31	19.32
Present study (women)	6.70	12.33

*Mean values calculated from authors' data.

task[9] or to the time between surgery and postoperative evaluations.[15]

Maximum Intensity Range. The maximum intensity range (loudest production minus softest production) increased significantly in this study. The authors are unaware of other reported findings for this variable.

Maximum Phonation Time. Maximum phonation time is a popular measure used to compare prethyroplasty versus postthyroplasty results. Even though the data collection and analysis procedures have varied — different vowels (/a/ versus /i/) or different instruments for analysis (digital stopwatches versus software application programs) — all investigators have reported a significant increase in maximum phonation time after thyroplasty (Table 5[9,12,14-17,19]). The findings from the present study are comparable with past reported findings. In the present study, the male values resulted in an average gain of 11 seconds, representing the highest gain among reported values.

AERODYNAMIC ASSESSMENT RESULTS

Glottal Airflow. The present findings, as well as past reported findings,[9,11,16,19] suggest that estimated glottal airflow significantly decreases after medialization thyroplasty. Bielamowicz et al[11] included some patients who underwent arytenoid adduction in addition to thyroplasty type I. Only the present study separated glottal airflow findings by sex. Normative data have indicated that airflow values are sex-dependent[26]; therefore, a comparison with studies that combined male and female data should be done cautiously.

Subglottal Pressure and Sound Pressure. The subglottal air pressure values did not change significantly as a result of thyroplasty in this study, but the sound pressure values recorded during the aerodynamic measurements increased for both the men and the women, with the increases being statistically significant for the men. Bielamowicz et al[11] reported similar subglottal pressure findings in 59 patients (20 women; 39 men), but did not report sound pressure values. Adams et al[9] reported a significant decrease in estimated subglottal pressure after thyroplasty in 13 patients (10 women; 3 men). In the Adams et al study, preoperative and postoperative sound pressures were controlled by having the subjects monitor their intensity level during the aerodynamic tasks, but values were not reported.

The lack of a significant postoperative change in subglottal pressure (in fact, average pressures were elevated both before and after surgery) might reflect persistent abnormalities in underlying vocal mechanisms and/or a persistence of preimplant hyperfunctional behavior. However, because of the relationship between subglottal pressure and vocal intensity (sound pressure), any discussion of a change in subglottal pressure should take into account changes in vocal intensity. The findings indicated that within each group, most patients were able to produce equally loud or louder voices, with approximately the same subglottal pressure, after surgery. This general observation was supported by the results of sound pressure and subglottal pressure comparisons, which reflected postsurgical increases in vocal efficiency; ie, men and women displayed an increase in the sound pressure–subglottal pressure ratio, with the changes in both female and male values being statistically significant. These findings suggest that the implant was effective in medializing the IVF and, therefore, improved the patient's ability to more efficiently produce sound.

CONCLUSIONS

The surgical advantages that have been described, and the findings that phonatory outcomes were comparable to those of previous assessments after medialization thyroplasty, support the view that the Montgomery® Thyroplasty Implant System appears to be an attractive alternative for treating unilateral vocal fold immobility. Suggestions for future studies include the use of imaging methods, such as magnetic resonance imaging, to better determine the placement and the size of the implant needed for individual patients, and examining the effect of voice therapy in addition to medialization thyroplasty type I with the Montgomery® Thyroplasty Implant System for patients with vocal fold immobility, especially when persistent hyperfunctional behavior is suspected to contribute to postimplant dysphonia.

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PATTERNS OF CHROMOSOMAL ABERRATIONS IN METASTASIZING AND NONMETASTASIZING SQUAMOUS CELL CARCINOMAS OF THE OROPHARYNX AND HYPOPHARYNX

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Although several cytogenetic events of the tumor progression cascade have been identified in the past, the specific types of chromosomal alterations that lead to the development of lymph node metastases are still unknown. Operative specimens of 20 patients (10 patients with metastasizing tumors, 10 patients with nonmetastasizing tumors) with squamous cell carcinomas of the oropharynx and hypopharynx, along with the corresponding lymph node metastases, were investigated by quantitative DNA measurements and comparative genomic hybridization (CGH). Nonmetastasizing tumors (N0) displayed overrepresentations on chromosomes 10q (8 cases); 5p (7 cases); 3q and 20q (6 cases each); 8q (5 cases); 1p and 21q (4 cases each); 7p and 20p (3 cases each); and 2p, 15q, and 19q (2 cases each). Loss of chromosomal material was found on 5q, 9p, and 14q (2 cases each). Metastasizing tumors (N+) demonstrated overrepresentations on chromosomes 5p, 15q, and 22q (6 cases each); 3q and 11q13 (5 cases each); 20p and 21q (4 cases each); and 10q (3 cases). In 2 cases, an overrepresentation of the chromosomal arm 3q was accompanied by a loss of chromosomal arm 3p. Less frequent overrepresentations were observed on chromosomes 1q and 17q. Deletions were found on chromosomes 18q (3 cases), 3p, 4q, 5q, and 19p (2 cases each); and sporadic deletions occurred on 2q, 6q, 8p, 9p, 10p, 13q, 14q, 15q, and 16q. Whereas overrepresentations on chromosomes 1p and 7p occurred exclusively in N0 tumors, overrepresentations on chromosomes 1q, 11q, and 22q, along with deletions on 18q, were only observed in N+ tumors. Quantitative DNA measurements revealed a significantly higher percentage of aneuploid cells and a higher degree of DNA entropy in the N+ tumors. Chromosomal overrepresentations on chromosomes 1q, 8q, 11q, 18q, and 19q occurred more frequently in the metastases than in the corresponding primary tumors. Pairwise analysis of chromosomal alterations in the primary tumors and associated lymph node metastases revealed a genetic relationship, although a greater number of chromosomes on average were affected in the lymph node metastases. Quantitative DNA measurements demonstrated greater aneuploid values in the metastases. Recurring patterns of chromosomal alterations in N0 and N+ tumors were demonstrated in this study. In general, metastasizing tumors are characterized by overrepresentations on chromosomes 11q13 and 22q, and deletions on 18q. These aberrations suggest an elevation along the tumor progression cascade.

KEY WORDS — chromosomal alterations, comparative genomic hybridization, hypopharynx, lymph node metastases, metastasizing and nonmetastasizing tumors, oropharynx, progression cascade, quantitative DNA measurements, squamous cell carcinomas.

INTRODUCTION

Squamous cell carcinoma is the most common malignant tumor occurring in the upper aerodigestive tract. Mutations induced by genotoxic agents, including aromatic hydrocarbons and nitrosamines of tobacco smoke, and by viral particles such as human papillomavirus have been considered as main sources in the development of cancer. It is probable that a variety of tumorigenic genetic mutations are required to initiate the cascade of events required in the alteration of benign mucosa to the development of a malignant tumor within an infiltrative growth pattern and lymph node metastases. During the past several years, various molecular, biological, and cytogenetic data on squamous cell carcinoma of the head and neck have been evaluated, and some of the specific cytogenetic events in the progression cascade have been identified.[1-4] However, recurring patterns of distinct cytogenetic aberrations in the cascade of events separating benign mucosa from malignant disease with lymph node metastases currently remain unclear.

Comparative genomic hybridization (CGH) is a technique to analyze unbalanced numeric and structural chromosomal aberrations from the whole genome of tumor cells.[5,6] To date, most studies on CGH in squamous cell carcinomas have been performed on cell cultures. Very little has been published comparing the chromosomal aberrations occurring in primary metastasizing tumors and the corresponding lymph node metastases and nonmetastasizing tumors by means of CGH on fresh tumor tissue. The purpose of the present study was to analyze cytogenetic aber-

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TABLE 1. TUMOR SITES AND TNM STAGE (UICC 1987) OF PATIENTS WITH NONMETASTASIZING TUMORS (N0) AND METASTASIZING SQUAMOUS CELL CARCINOMAS (N+)

	N0 Tumors (n = 10)	*N+ Tumors (n = 10)*
Gender		
Female	2	1
Male	8	9
Age (y)	56.8 (range 44 to 69)	50.8 (range 41 to 65)
TNM stage		
T1	3	2
T2	4	4
T3	2	3
T4	1	1
N0	10	
N2a		3
N2b		5
N2c		2
M0	10	10
Tumor site		
Oropharynx	6	6
Hypopharynx	4	4
Histology		
Moderately well differentiated	8	7
Poorly differentiated	2	3

rations in primary tumors, lymph node metastases, and nonmetastasizing tumors to identify a recurring pattern of aberrations or specific aberrations leading to the development of lymph node metastases.

PATIENTS AND METHODS

Operative specimens of 20 patients (17 men, 3 women) with an average age of 53.8 years (range 41 to 69 years) who underwent surgery for squamous cell carcinoma of the oropharynx or hypopharynx were examined for this study.

The patients were divided into 2 groups. The first group comprised patients with nonmetastasizing tumors (N0; n = 10). Lymph node metastases were excluded in this group by preoperative clinical and ultrasound examinations and histologic examination of the neck dissection tissue. The second group comprised patients with metastasizing tumors (N+; n = 10). All of these patients underwent neck dissection together with the excision of the primary tumor, allowing for an evaluation of both the primary tumor and the metastatic node(s).

The tumor sites and TNM stages of all patients are summarized in Table 1. Examinations performed included routine histology and quantitative DNA analysis, along with CGH.

Routine Histology and Quantitative DNA Analysis. All tumors were radically excised with histologically clear margins confirmed by serial sections. Ipsilateral neck dissection was also performed, and the metastases were histologically confirmed in the second group. None of the neck tissue from patients of the first group contained metastatic nodes.

A specimen from each tumor was immediately placed in liquid nitrogen. By means of a cryostat, 6-μm, 20-μm, and 50-μm slices were obtained. The 6-μm sections were stained with hematoxylin and eosin for the morphological examinations.

Quantitative DNA analysis was performed on cytologic smears obtained from the 50-μm slices. The tumor tissue was mechanically disaggregated by pressing the tissue through a syringe with a 0.9 × 70-mm needle. The material was enzymatically disaggregated as well, by incubation in 0.05% pronase at 37°C for 30 minutes.[7,8] The suspension was then washed in distilled water and centrifuged by cytospin onto a glass slide at 3,000 rpm. The slides were then air-dried, fixed with isopropanol, and stained with fuchsin–sulfuric acid according to Feulgen and Rossenbeck.[9]

Quantitative DNA measurements were obtained with a computerized television-based image analysis system (Cytometer CM1, Hund Cie, Wetzlar, Germany). At least 300 tumor cells were measured at random after detection on the television screen. The normal 2c peak was determined by evaluating at least 25 lymphocytes in the same smear. These cells functioned as reference cells. The strength of this kind of image analysis lies in the opportunity for mathematical processing of the optical images to get quantifiable nuclear DNA parameters. The following indices were derived from the single cell measurements[10] of the mean DNA content of the tumor cells.

1. The 2c deviation index (2cDI) — the variance of the DNA content from 2c. It is defined as the sum of squares of the differences between the DNA values of single cells (ci) and the 2c value divided by the number of measured cells[11]:

$$2\text{cDI} = \frac{1}{N} \times \sum_{i=1}^{N} (\text{ci} - 2\text{c})^2$$

2. The 5c exceeding rate (5cER) — the percentage of cells with an aneuploid DNA content exceeding 5c. This index is computed from the single cells with a DNA content exceeding 5c. A 5cER of 10%, for example, means that 10 out of 100 tumor cells measured have a DNA content beyond 5c.[11]

3. Entropy — a measure of the variation of the DNA content of all cells. It is defined as the irregularity of the DNA distribution in 80 channels with a

class width of 0.1c each.[12]

4. The DNA malignancy grade (DNA-MG) — computed from the 2cDI values as a logarithmic transformation to get a malignancy grade for clinical use. According to Boecking,[10] it is computed by the formula DNA-MG = 1.748 × log(2cDI + 1). The DNA-MG ranges from 0.01 to 3.0, with 0.01 representing benign disease and 3.0 signifying the most malignant state.

Comparative Genomic Hybridization. To isolate tumor DNA, 20-μm sections were cut from the frozen tumor tissue and stained with methylene blue. Tumor islands of 100 to 200 cells were obtained by microdissection (Micromanipulator, Carl Zeiss, Jena, Germany). The samples were incubated in 100-mmol/L Tris–hydrochloric acid (pH 8.0), 10-mmol/L ethylenediaminetetraacetic acid, and 200-mmol/L sodium chloride + 1% sodium dodecyl sulfate + 500-μg/mL proteinase K overnight at 37°C. The supernatant was extracted repeatedly with PCL (phenol, chloroform, and isoamyl alcohol). Control DNA was isolated from 1 mL of fresh heparinized blood. Degenerated oligo priming polymerase chain reaction (DOP-PCR) was performed according to the technique of Telenius et al.[13] Only DOP-PCR reactions showing a distribution of DNA fragments with a maximum frequency in size between 1 and 2 kilobase were used for subsequent DNA labeling reactions via nick translation. Tumor DNA was labeled with biotin-11-desoxy-uridine triphosphate, and control DNA was labeled with digoxigenin-11-desoxy-uridine triphosphate (Boehringer-Mannheim, Germany). The ratio of DNA polymerase to DNase I was adjusted empirically to achieve a fragment size range of hapten-labeled DNA from 100 to 700 base pair (bp; maximum frequency of 300 bp).

To perform the CGH, 1 μg of both digoxigenin-labeled control DNA and biotin-labeled tumor DNA were coprecipitated in the presence of 70 μg Cot1-DNA (Gibco BRL Life Technologies, Eggenstein, Germany) in ethanol. Precipitated DNA was resuspended in 5 μL of deionized formamide, agitated for 1 hour at room temperature, and fixed on a vortex before 5 μL of a hybridization buffer (4× SSC, 20% dextran sulfate in a sodium phosphate buffer [pH 7.0]) was added. The solution was then agitated again for 30 minutes, denatured at 70°C for 5 minutes, transferred directly to a moist chamber, and preannealed for 30 minutes at 37°C.[5,14]

Metaphase chromosomes were prepared and spread on glass slides. After denaturation of the chromosomes, the glass slides were prewarmed at 32°C and the hybridization mixture was applied. An 18 × 18-mm coverslip was mounted onto the region of interest, with care taken to avoid air bubbles, and then sealed with rubber cement. The hybridization procedure was performed in a moist chamber at 37°C for at least 36 hours.

The slides were stained with anti-digoxigenin-mab-fab-rhodamine and streptavidin–fluorescein isothiocyanate (FITC; Boehringer-Mannheim), 5 μg/mL each in 200 μL SSC detection solution (4× SSC 1%, bovine serum albumin 0.1%, Tween 20) for 30 minutes at 37°C. The chromosomes were counterstained with 50 ng/mL DAPI (4',6'-diamidino-2-phenylindole) in 2× SSC.

Chromosomes showing signals in the CGH experiments were identified by karyograms of DAPI-stained metaphase chromosomes by means of a software package (AKS software, version 3.2.1, Vysis Inc, Stuttgart, Germany). Digital fluorescence microscopy was performed with a cooled chip device (CCD) camera (Photometrix Kodak KAF 1400, Kodak, Tucson, Ariz) mounted on an epifluorescence microscope (Axioskop, Zeiss, Oberkochen, Germany). Three fluorescence images (FITC, rhodamine, DAPI) per metaphase were acquired.

Digital image acquisition and digital image analysis were performed by means of software packages (IPLab 2.5.7 and Quips 1.0; Vysis Inc) running on an Apple Macintosh Power PC 8100/100. The filter sets for DAPI, rhodamine, and FITC were integrated in a Ludel filter wheel and controlled by the software (Fig 1).

Data from at least 10 metaphases were averaged in each case. If the mean fluorescence ratio of the FITC and TRITC images corresponding to the theoretical value of a monosomy in 50% of the tumor cells was below 0.5 + 0.1 (1 SD), it was considered a deletion. If the fluorescence ratio exceeded 1.5 – 0.1, it was considered an overrepresentation.[15]

RESULTS

Quantitative DNA Analysis. Quantitative DNA analysis revealed aneuploid tumor cells with a DNA content exceeding 5c in every tumor. The results are summarized in Table 2. In the comparison of metastasizing and nonmetastasizing tumors, the values for mean DNA content of the tumor cells, 2cDI, 5cER, and entropy were significantly higher in the N+ tumors than in the N0 tumors, indicating a higher degree of aneuploidy and a more aggressive behavior of the tumor cell clones in the N+ tumors. Figure 2 gives examples of DNA histograms of metastasizing and nonmetastasizing tumors. The highest values, however, were observed in the tumor cells from the lymph node metastases.

Cytogenetic Aberrations in Metastasizing and Non-

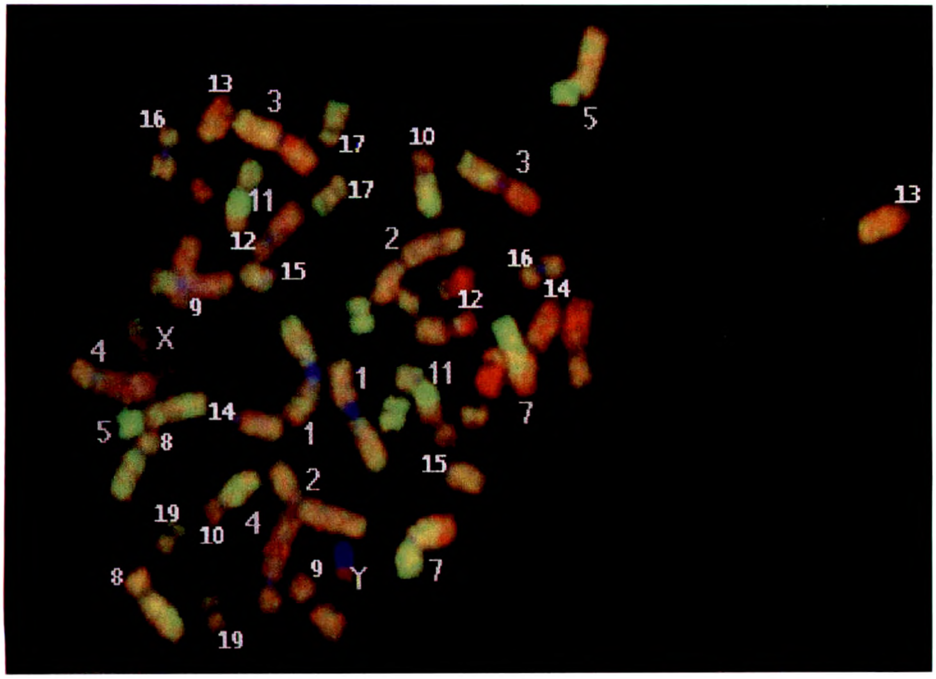

Fig 1. Comparative genomic hybridization experiment with fluorescein isothiocyanate–labeled tumor DNA obtained from fresh tumor tissue and rhodamine-labeled DNA obtained from normal lymphocytes. Parts of chromosomes with overrepresentations show green color, and parts of chromosomes with deletions show brownish color.

metastasizing Tumors. The most frequent cytogenetic aberrations in N0 tumors were found as overrepresentations on chromosomes 10q (8 of 10 cases); 5p

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with isolated or sporadic deletions on chromosomes 2q, 6q, 8p, 9p, 10p, 13q, 14q, 15q, and 16q. On average, more chromosomes were affected in metasta-

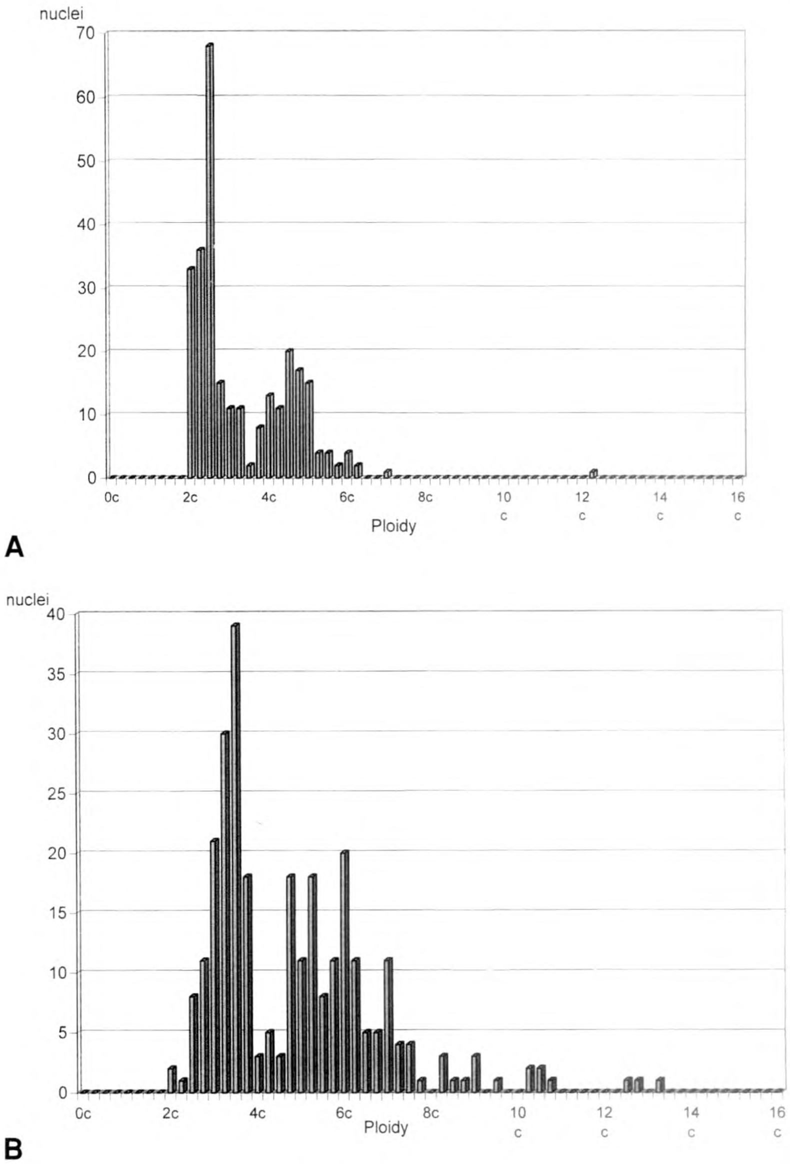

Fig 2. Examples of quantitative DNA analysis in A) nonmetastasizing (N0) and B) metastasizing (N+) tumors. N+ tumor displayed numerous cells with aneuploid DNA content exceeding 5c (5c exceeding rate: 27.3%). 2cDI is 9.85 and entropy value is 4.92 in this case. N0 tumor showed 5cER of 9.7%, 2cDI of 3.8, and entropy value of 4.02.

ses. In 3 tumors, the overrepresentation of the chromosomal arm 3q was accompanied by a loss of chromosomal arm 3p. Frequent overrepresentations occurred on chromosomes 19q and 20p (5 cases each); 5p, 10q, and 17q (4 cases each); and 8q and 18q (3 cases each). Sporadic overrepresentations were observed on chromosomes 17p (2 cases) and 2p, 4q, 6p, 9q, 13q, 16p, and 20q (1 case each). Frequent chromosomal losses were observed on chromosomes 3p and 11p (3 cases each) and 4p, 5p, 9p, and 13q (2 cases each). Sporadic deletions occurred on chromosomes 6p, 10p, 14q, 15q, and 19q (1 case each).

Chromosomal losses on chromosome 11p and overrepresentations on chromosome 17p were observed in tumor cells of lymph node metastases, but not in primary metastasizing tumors. Overrepresentations on chromosomes 1p, 8q, 11q, 18q, and 19p occurred more frequently in tumor cells of lymph node metastases when compared to the primary tumors. Overrepresentations on chromosomes 21q and 22q occurred with similar frequency in primary tumors and lymph node metastases.

Pairwise analysis revealed that the following pattern of aberrations could be observed in both the primary tumors and the corresponding lymph node metastases: gain of chromosomal material on chromosomes 22q (6 cases), 5p, 11q, and 15q (4 cases each), 3q, 10q, 20p, and 21q (3 cases each), 19p and 17q (2 cases each), and 8q, 9q, 14q, and 18q (1 case each); and deletions on chromosomes 3p, 5q, 11p (2 cases each) and 13q, 18q, and 19q (1 case each). This correspondence indicates the genetic relationship between lymph node metastases and primary tumors (Fig 4).

DISCUSSION

It is widely accepted that the expression of an aneu-

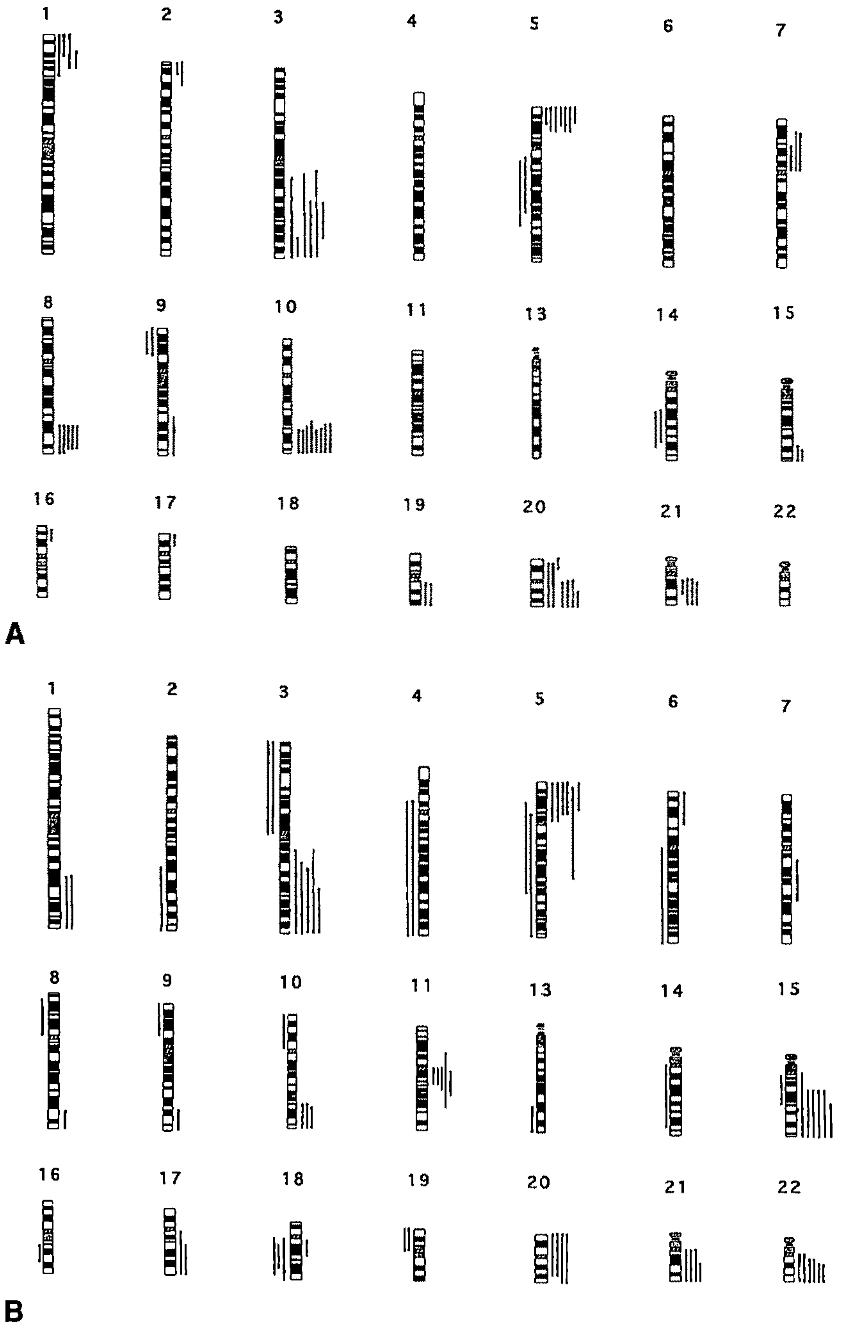

Fig 3. Comprehensive view of chromosomal aberrations in A) N0 and B) N+ tumors. Gains of chromosomal material are marked by bars on right side of chromosomes. Overrepresentations on chromosomes 1p and 7q exclusively occurred in N0 tumors. Overrepresentations on chromosomes 1q, 11q, and 22q, along with deletions on chromosome 18q, could only be observed in N+ tumors.

ploid DNA content is characteristic of malignant cells. Several cytogenetic aberrations that occur during carcinogenesis of squamous cell carcinomas of the head and neck have been identified recently.[16,17] Nevertheless, the particular molecular and cytogenetic events leading to aneuploidy, tumor progression, and ultimately the development of lymph node metastases remain unclear.

Comparative genomic hybridization is a technique used to detect unbalanced chromosomal aberrations in the whole genome of tumor cells.[6] One advantage of this method is that it screens for chromosomal gains and losses and provides information about the chromosomal sites in which they occur.[18]

In the present study, we investigated the specific and recurring chromosomal aberrations in nonmetastasizing (N0) tumors, metastasizing (N+) tumors, and corresponding lymph node metastases of squamous cell carcinomas of the oropharynx and hypopharynx. Cells for the CGH experiments were obtained from frozen tumor tissue by microdissection so that particular tissue regions with a high percentage of tumor cells could be chosen to provide some morphological control of the cells examined. This qualitative genetic analysis of tumor cell genomes, in combination with quantitative DNA cytometry, should permit an inte-

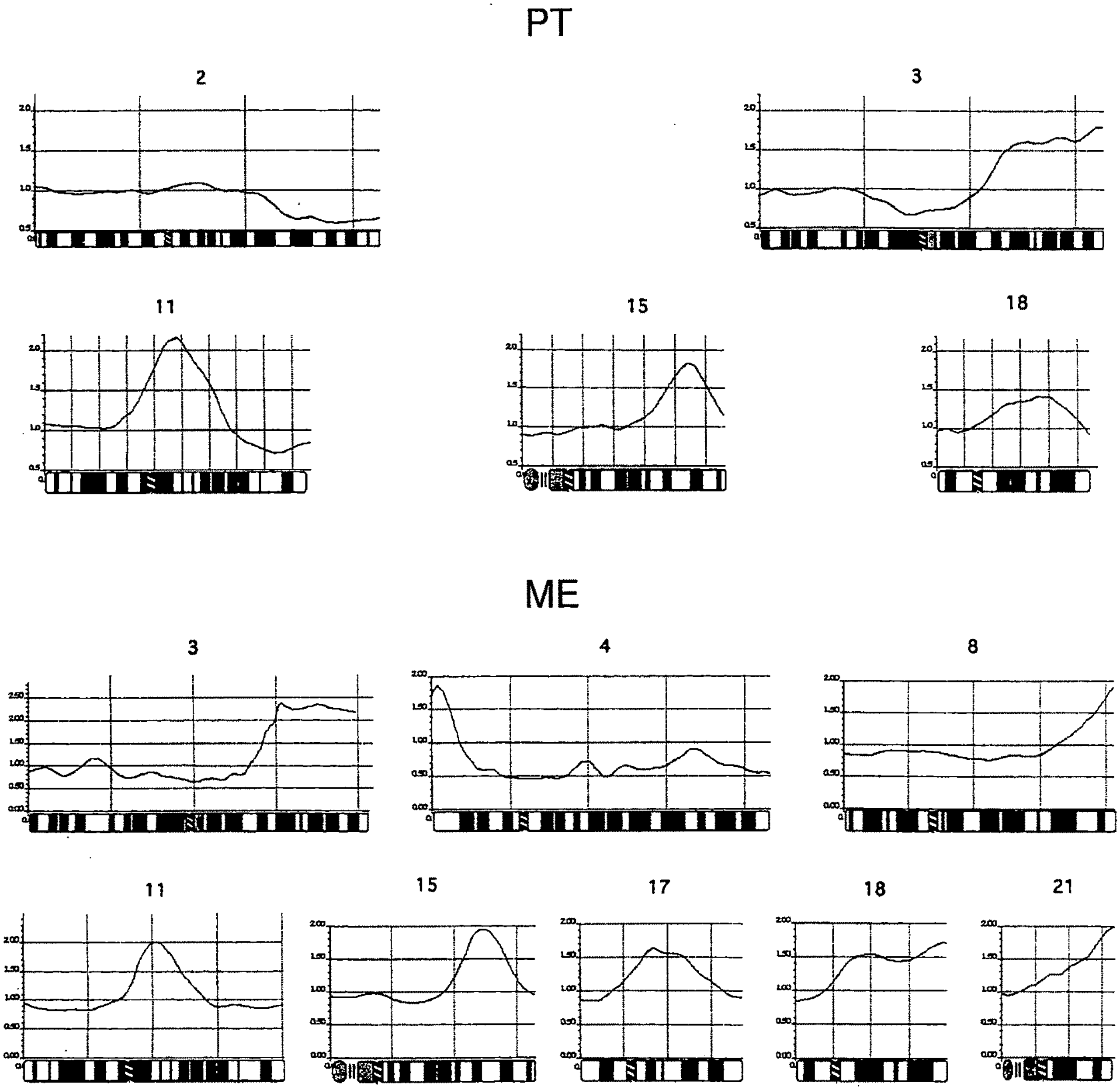

Fig 4. Comparison of averaged epifluorescence ratios in primary tumor (PT) and in corresponding lymph node metastasis (ME). In chromosomes 3, 11, 15, and 18, similar signals occurred.

grated view of their genomic constitution.

Patterns of Chromosomal Aberrations in N0 and N+ Tumors. One might expect that the development of lymph node metastases reflects a higher stage of the tumor progression cascade. Recently, Sidransky[16] and Califano et al[19] proposed a model of the progression cascade in squamous cell carcinomas that indicates that deletions on chromosomes 9p21, 3p14, and 17p are early events in tumor development, as they are also observed in premalignant epithelial lesions such as epithelial dysplasias.[20] Chromosome 9p21 contains the locus of the cell cycle gene p16, which is an inhibitor of the cyclin-CDK complex. Additional amplifications on chromosome 11q13 and deletions on chromosomes 14q and 13q should be regarded as basic events for the development of invasive cancer and aggressive behavior.

In general, we found more chromosomes affected by aberrations in N+ tumors than in N0 tumors. The aberrations observed in N0 tumors were overrepresentations on chromosomes 10q, 20q, 8q, 3q, 1p, and 21q and deletions on chromosome 9p. The most frequent aberrations in N+ tumors were overrepresentations on chromosomes 11q13, 5p, 15q, 22q, 10q, 21q, 20p, and 3q and losses of chromosomal material at 18q, 3p, 19p, 4q, and 5q. It is important to note that overrepresentations of 11q13 and 22q were observed in N+ tumors, but not in N0 tumors. Additionally, deletions on chromosome 18q occurred only in N+ tumors, whereas amplifications on 7p occurred only in N0 tumors. Gains of chromosomal material on 15q were more frequent in N+ tumors, with overrepresentations on 10q and 20q occurring more frequently in N0 tumors. DNA cytometry revealed significantly higher percentages of aneuploid tumor cells

myeloid leukemias, deletions on 20q11 were observed.[30] Other studies revealed that chromosome 20 is highly involved in radiation-induced structural chromosome aberrations.[31]

We did not find a high incidence of deletions of 5q and 13q, in contrast to other studies.[32] One reason

[redacted]

metastases, we discovered recurring patterns. Overrepresentations on 20p, 21q, and 22q occurred with comparable frequency, as well as deletions on 18q, indicating a genetic relationship between tumor cells in metastases and those in the primary tumor. Overrepresentations, which were more frequent in lymph node metastases, were identified on 1q, 8q, and 17q. Thus, the tumor cells in metastases may undergo an autonomous development leading to an accumulation of genetic aberrations, ultimately resulting in a different biological and clinical behavior than that observed in the primary tumor.[36,37] Alternatively, metastases may derive from distinct cell clones within the primary tumor, which have a higher degree of genetic abnormalities.

In conclusion, we were able to demonstrate recurring chromosomal alterations in nonmetastasizing and metastasizing squamous cell carcinomas of the oropharynx and hypopharynx, as well as recurring chromosomal alterations in corresponding lymph node metastases. Metastasizing tumors seem to be characterized by additional cytogenetic aberrations such as overrepresentations on 11q13 and 22q and deletions on 18q. These tumors have a significantly elevated percentage of aneuploid tumor cells detected by quantitative DNA cytometry. These data should increase our understanding of the development of lymph node metastases and may enhance our ability to perform molecular tumor staging. Hopefully, further study will allow us to predict the likelihood of metastatic spread in the clinically negative neck on the basis of information obtained from the primary tumor. Certainly, more studies are required to identify additional chromosomal locations for possible tumor suppressor genes.

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EUROPEAN ACADEMY OF FACIAL PLASTIC SURGERY

The annual meeting of the European Academy of Facial Plastic Surgery will be held September 15-17, 2000, in Thessaloniki, Greece. For information, contact Prof Dr J. Daniilidis, ENT Dept, Aristotelian University of Thessaloniki-AHEPA Hospital, 540 06 Thessaloniki, Greece; telephone 0030/31/994.761-2; fax 0030/31/994.916.

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PEDIATRIC LYMPHANGIOMAS OF THE HEAD AND NECK

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To better define the cause, presentation, diagnosis, and staging of lymphangiomas, we reviewed all cases of lymphangiomas of the head and neck region in children seen at our institution between 1986 and 1996. Sixty-seven children were identified (31 male and 36 female). Age at presentation ranged from birth to 18 years. All but 8 patients presented with an obvious mass, and 5 required tracheotomy because of airway obstruction. The most common location was the submandibular region (37%), followed by the parotid gland (31%). Treatment ranged from observation to extensive and multiple resections. Thirty-one patients underwent only 1 resection, and 2 patients received interferon as part of their treatment. Lesions involving the lip, hypopharynx and/or larynx, the tongue, and the floor of the mouth had high rates of recurrent or persistent disease. We review our experience with these difficult lesions and propose a staging system based on functional deficit, cosmetic changes, sites of involvement, and age at diagnosis.

KEY WORDS — cervicofacial malformations, congenital malformations, head and neck tumors, lymphangioma, lymphovenous malformations.

Lymphangiomas are unusual lesions that are believed to develop as a result of a congenital malformation of the lymphatic system[1,2]; however, there is no agreement as to the reason these malformations occur. The lymphatic system forms during the sixth week of embryonic development from paired jugular sacs that began as clefts in the cervical mesenchyme. Three theories exist to explain the origin of lymphangiomas. One is that the primordial lymphatic sacs fail to connect with, and thus drain into, the venous system.[3] The second theory is that lymphangiomas are a result of lymph tissues that are deposited in the wrong area during embryogenesis and subsequently fail to join the normal lymph system.[3] Third, these lesions have been attributed to abnormal budding of the lymphatic system and a loss of connection to the central lymph channels.[4]

Regardless of their cause, these tumors are uncommon, composing only 5.6% of all benign lesions in children.[5] Most often located in the head and neck region, these lesions are prone to sudden and extensive enlargement and can produce considerable cosmetic and functional impairment. Described as soft, nontender masses, these lesions often are classified as capillary lymphangiomas, cavernous lymphangiomas, and cystic hygromas on the basis of their histologic appearance.[6] Because the lesions often have a mixture of these subtypes, it has been proposed that these subtypes most likely represent the same entity and that their histologic features are governed by the nature of the surrounding tissue rather than by inherent biological behavior.[7] Given that several histologic subtypes can exist within a single lesion and the common presence of a vascular component, they are unlikely to regress spontaneously. Jackson et al[8] grouped these lesions with other vascular malformations characterized as low-flow mixed lymph and venous lesions.

A lymphangioma is diagnosed on the basis of the clinical history, physical examination, and radiologic evaluation. Although considered a benign lesion, this malformation can be extensive and may invade or impinge on surrounding structures. Treatment options include surgical excision, laser debulking, sclerotherapy, and corticosteroids, and therapy must be individualized to provide optimal results. The desire for complete excision must be weighed against the need to keep vital structures intact, and often, treatment results in incomplete excision and subsequent recurrence. In an attempt to formulate a more consistent evaluation and treatment plan, we reviewed all cases of cervicofacial lymphangiomas managed at our institution in the past 10 years. Using this information, we suggest a staging system for these lesions and discuss related treatment options.

PATIENTS AND METHODS

The medical records of all children (younger than 19 years) with a diagnosis of lymphangioma made at the Mayo Clinic between 1986 and 1996 were reviewed retrospectively. Excluded from this study were patients with superficial, vesicular, cutaneous lymphangiomas (lymphangioma circumscriptum) and patients with lymphangiomas outside the cervicofacial area. The records of the patients who fulfilled the study criteria were reviewed to determine age at diag-

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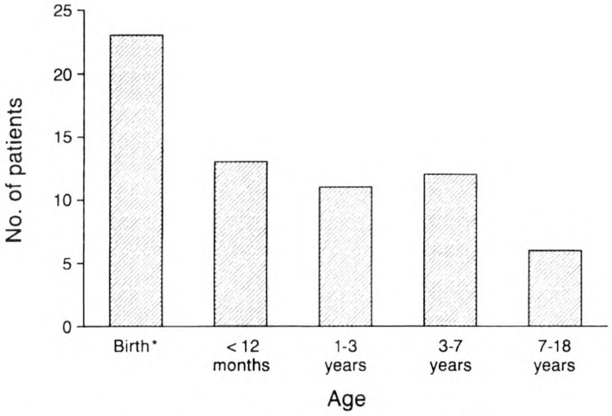

Fig 1. Age at diagnosis of lymphangiomas in 65 patients. * — Includes 1 patient with prenatal diagnosis.

nosis, sex, presenting symptoms, size and location of lesion, diagnosis, treatment, recurrence, complications, and outcome. All patients who had not been seen in follow-up within the past 3 months received follow-up telephone interviews to establish the status of the lesion, its appearance, and its effect on daily function. These results were analyzed statistically.

RESULTS

Patients. The retrospective review identified 67 patients: 36 (54%) were female and 31 (46%) were male. Two of the patients (both male) had been stillborn before the third trimester of pregnancy. Both patients had large lesions involving the posterior aspect of the neck and occiput and multiple congenital anomalies. One was karyotypically normal, and the other had trisomy 21. These 2 patients were not included in the statistical analysis. Of the remaining 65 patients, their ages at diagnosis ranged from birth to 18 years. The 1 patient who had the lesion identified before birth was grouped with patients whose lesions were diagnosed at birth. The diagnosis was made at birth in 23 of the 65 patients (35%), within the first year after birth in 36 (55%), and within the first 3 years in 47 (72%); the initial diagnosis was made between the ages of 10 and 18 years in only 6 patients (9%; Fig 1). There was minimal association between these lesions and other congenital abnormalities; 2 patients had Klippel-Trénaunay syndrome (3%; Fig 2), one had Proteus syndrome (2%), and 1 was born with a coloboma (2%). One patient also had a second branchial cleft cyst removed at the time of excision of the lymphangioma.

Symptoms. The most common presenting symptom was a noticeable mass in 57 (88%) patients; the 8 other patients had intraorbital lesions and no obvious mass. Thirty-six patients (55%) had an increase in the size of the mass with upper respiratory tract infection, 10 (15%) experienced proptosis, 8 (12%) had pain, and 8 (12%) experienced some degree of respiratory distress. Of the 8 patients with respiratory distress, 7 (88%) had involvement of the hypopharynx and/or larynx, 5 (63%) had involvement of the tongue, and 4 (50%) had involvement of the oropharynx. (Five patients had more than 1 site of involvement.) Five (63%) of the 8 patients with respiratory distress required tracheotomy at some time after diagnosis.

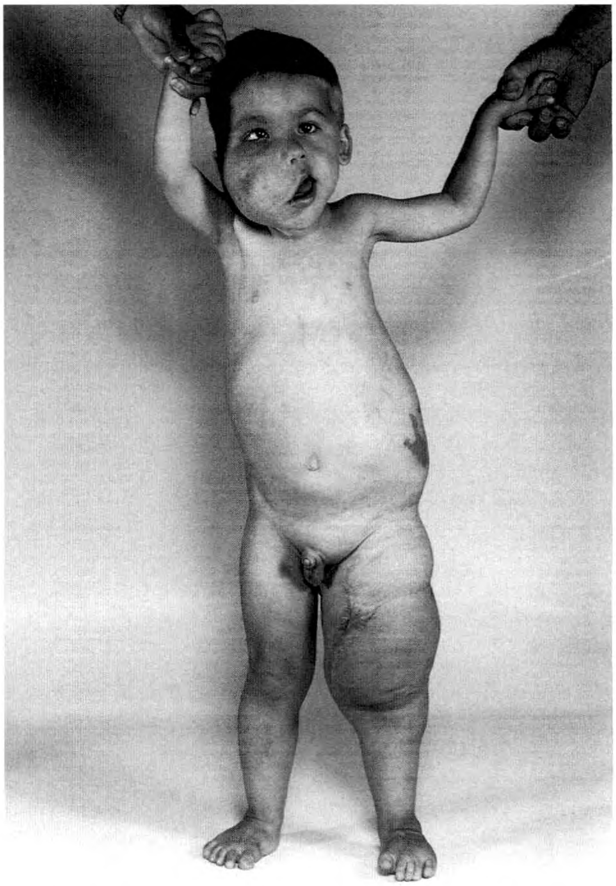

Fig 2. Patient with lymphangioma and Klippel-Trénaunay syndrome.

Other symptoms included dysphagia in 6 patients (9%), and 3 (5%) exhibited dysphonia. All but 1 of these patients had lymphangioma involving the upper aerodigestive tract, including the oropharynx (4 patients), tongue (4 patients), or hypopharynx and/or larynx (3 patients); 1 patient had extensive involvement of the parapharyngeal area that resulted in some dysphagia.

Diagnosis. The diagnosis of lymphangioma was based on the history and physical examination alone, on these findings with the addition of radiologic evaluation, or on the basis of all 3 evaluations and pathologic diagnosis. Fifty-two patients (80%) had tissue taken at the time of operation that confirmed the diagnosis of lymphangioma. Of the 13 other patients

TABLE 1. LOCATION OF LYMPHANGIOMAS WITHIN HEAD AND NECK IN 65 PATIENTS

Location	*No. of Patients**	*% of Patients**
Submandibular	24	37
Parotid gland	20	31
Anterior neck	15	23
Cheek (buccal space)	15	23
Tongue	13	20
Orbit	10	15
Parapharyngeal space	10	15
Posterior neck	8	12
Oropharynx	8	12
Hypopharynx and/or larynx	7	11
Infratemporal fossa	6	9
Floor of mouth	4	6
Supraclavicular	3	5
Lip	3	5
Nasopharynx	2	3
Tonsil	2	3
Mediastinum	1	1.5
Intracranial	1	1.5

*Thirty patients (46%) had more than 1 location involved with tumor.

who underwent no surgical therapy, the diagnosis was based on the behavior of the lesion (such as swelling at the time of upper respiratory tract infection) and on its appearance both clinically and on radiologic studies. Four (31%) of these 13 tumors were intraorbital. Imaging was performed to confirm the diagnosis and to evaluate the extent of the lesion in 54 (83%) patients. Computed tomography was the most common study performed. Thirty-five (54%) of our patients underwent computed tomography as part of their evaluation, 15 (23%) had magnetic resonance imaging, 10 (15%) had ultrasonography, and 1 was evaluated with magnetic resonance angiography to rule out arteriovenous fistula. Thirteen patients (20%) underwent more than 1 type of evaluation, and 11 patients (17%) were not evaluated radiologically.

The lymphangiomas had no predilection for a side of involvement: 48% occurred primarily on the right side of the head or neck, and 46% occurred primarily on the left. Four tumors (6%) occurred primarily in the midline. The most common location involved with lymphangioma was the submandibular area, in 24 patients (37%), followed by the parotid gland (31%) and the cheek (23%; Table 1). Thirty patients (46%) had involvement of more than 1 area simultaneously. The relationship between the number of locations involved with lymphangioma and the number of surgical procedures required is outlined in Table 2. Patients who had involvement in 3 or more areas of the head and neck were more likely to require multiple surgical interventions (exact Kruskal-Wallis p value, <.0001).

TABLE 2. RELATIONSHIP BETWEEN NUMBER OF LOCATIONS INVOLVED WITH LYMPHANGIOMA AND NUMBER OF SURGICAL PROCEDURES

	No. of Surgical Procedures		
No. of Locations Involved	*0*	*1*	*≥2*
1	12	20	3
2	3	7	1
3	0	3	1
4	1	1	5
≥5	0	0	8

Data are numbers of patients.

TABLE 3. RELATIONSHIP BETWEEN AGE AT DIAGNOSIS AND NUMBER OF SURGICAL PROCEDURES IN PATIENTS WITH LYMPHANGIOMAS

Age at Diagnosis	*No. of Patients (n = 65)*	*No. of Surgical Procedures*		
		0	*1*	*≥2*
Birth*	23	2	11	10
<12 mo	13	5	5	3
1 to 3 y	11	4	6	1
>3 y	18	5	9	4

Data are numbers of patients.

*Includes 1 patient with prenatal diagnosis.

Treatment. The treatment of the malformations was primarily surgical. Forty-nine patients (75%) underwent at least 1 surgical procedure, and 36 of these patients underwent surgery at our institution. The number of procedures for each patient ranged from 1 surgical excision in 31 patients (48%) to more than 10 surgical procedures in 4 patients (6%). These procedures ranged from simple local excision to extensive resections, depending on the size and location of the tumor.

The relationship between age at onset and the number of surgical procedures performed is shown in Table 3. There was a statistically significant trend toward the need for more surgical procedures in patients whose lymphangiomas were diagnosed at an early age (1 year of age or less; exact Kruskal-Wallis p value, .0411). Eight patients (12%) underwent carbon dioxide (CO_2) laser treatment for their lesion, and in 3 patients it was the only treatment they received. The laser was used to reduce lesions in the oral cavity and the upper airway. In no case was laser treatment used as a means of complete excision; rather, it was intended as a means of debulking the lesions. Other treatments included interferon in 2 patients (3%) and corticosteroid therapy in 2 patients (3%). Thirteen patients (20%) received no surgical or medical treatment and were followed with observation only.

Of the 13 patients who received no surgical or medical therapy, 12 had lymphangioma involving only 1 site and 1 patient had involvement of 3 sites

TABLE 4. LOCATION AND OUTCOME OF LYMPHANGIOMA IN 13 PATIENTS TREATED WITH OBSERVATION ONLY

Location	*Radiologic Study*	*Resolution*
Cheek	CT, US	Yes
Tongue	None	No
Anterior neck	CT	Yes
Cheek	CT	No
Orbit	CT	Yes
Parotid gland	CT	Yes
Tongue	None	No
Orbit	CT	No
Tongue	CT	No
Cheek	MRI	No
Orbit	MRI, US	No
Cheek	None	No
Cheek, parotid gland, infratemporal fossa	MRI	No

CT — computed tomography, US — ultrasonography, MRI — magnetic resonance imaging.

(Table 4). Ten (77%) of the 13 patients had radiologic evaluation of their tumor, including computed tomography, magnetic resonance imaging, and ultrasonography (Table 4). Four (31%) of the 13 patients reported eventual resolution of their lesion. These 4 lesions were located in the orbit, in the cheek, deep within the anterior neck, and within the parotid gland, and no radiologic studies were performed to confirm the complete resolution of the lesion. The remaining 9 patients reported persistent but stable disease at last follow-up.

Of the 65 patients, 21 (32%) had received surgical treatment elsewhere before evaluation at our institution. Of these 21 patients, 9 had further operations at our institution, and 3 were treated with a CO_2 laser as an attempt to further debulk their tumors. Among the 9 patients who underwent further operations at our institution, 6 (67%) had persistence of their lesion. Five of these 6 patients had lymphangioma that involved 2 or more sites and was extensive, and all 5 had involvement of the hypopharynx and/or larynx, oral cavity, or both. The sixth patient had a lesion involving only the submandibular region that recurred despite a second attempt at complete excision. Three of the 9 patients underwent complete excision of their tumor with no evidence of persistent disease. The remaining 9 patients who received surgical treatment elsewhere were followed with observation only.

TABLE 5. MOST COMMON LOCATIONS ASSOCIATED WITH PERSISTENT LYMPHANGIOMA

		Persistent Disease	
Location	*Total No. of Patients**	*No. of Patients*	*%*
Floor of mouth	4	4	100
Lip	3	3	100
Intracranial	1	1	100
Tonsil	2	2	100
Cheek	15	12	80
Hypopharynx and/or larynx	7	5	71
Oropharynx	8	5	63
Tongue	13	6	46

*Multiple sites are possible.

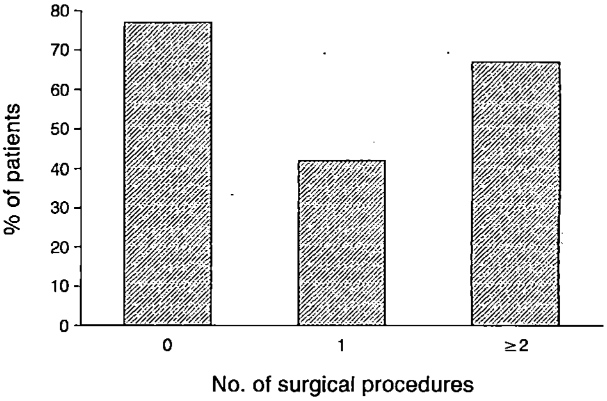

Fig 3. Relationship between number of surgical procedures and persistent disease in patients with lymphangiomas.

Thirty-six patients underwent their initial operation at our institution. Twenty-three underwent an attempt at complete surgical excision as a primary procedure. The recurrence rate in these patients was 22% (5 patients). The most common locations associated with incomplete removal or recurrence in these patients were the anterior (2 patients) and the posterior (2 patients) aspects of the neck. One patient had lymphangioma involving the parotid gland and cheek, and persistent tumor involved the cheek and buccal mucosa.

Because of the extent of the lesion and the risk to critical structures, 4 patients treated surgically at our institution underwent intentional partial excision or debulking of their tumor, and as expected, all had persistent disease. Three of these patients had orbital tumors, and the fourth had a huge lesion involving both sides of the neck, the oral cavity, the tongue, and the hypopharynx. This patient underwent multiple operations in an attempt to maintain the airway and decrease the size of the lesion.

Persistence. When only the 36 patients who had their initial surgery at our institution are considered, the number who had persistent disease at follow-up is 14 (39%). The most common sites of persistent disease in these patients were the tongue (6 patients) and the hypopharynx and/or larynx (5 patients). When all 65 patients are considered, the distribution of sites involved with recurrent disease is as outlined in Table 5, and the relationship between the number of surgical procedures and persistent disease is as shown in

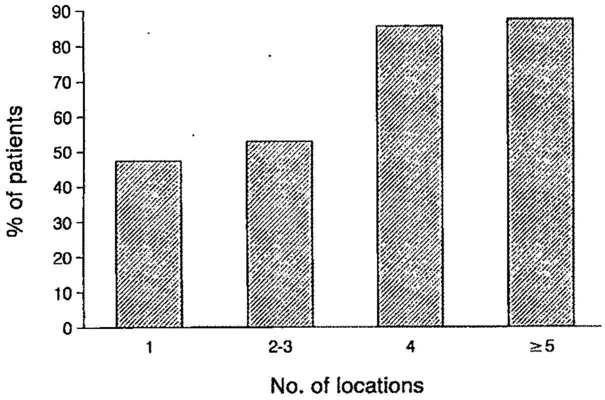

Fig 4. Relationship between number of locations involved with lymphangiomas and persistent disease.

Fig 3. In contrast, the site most likely to undergo complete excision was the submandibular area; 11 (46%) of the 24 patients who had involvement of this area had complete excision without recurrence. None of these 11 patients with lymphangioma involving the submandibular area had involvement of more than 3 areas, and 5 of them had involvement of the submandibular area alone. As expected, the more locations in the cervicofacial area involved with tumor, the higher the likelihood of persistent disease (Fig 4).

Complications. Major complications related to surgical excision included permanent facial nerve paralysis involving at least 1 major branch of the nerve in 8 patients (12%), hypoglossal nerve injury in 2 patients (3%), and accessory nerve injury in 1 patient (2%); 2 patients had injury to more than 1 cranial nerve. The overall incidence of permanent cranial nerve injury in the 49 patients who underwent operation was 20%. Minor postoperative complications included temporary facial weakness in 3 patients (5%), seroma formation in 5 (8%), hematoma formation in 3 (5%), and a significant chyle leak in 1 (2%). The chyle leak was considered a minor complication because it resolved spontaneously after many months of drainage. The distribution of these complications in relation to the number of surgical procedures performed is outlined in Table 6.

TABLE 6. RELATIONSHIP BETWEEN NUMBER OF SURGICAL COMPLICATIONS AND NUMBER OF SURGICAL PROCEDURES IN PATIENTS WITH LYMPHANGIOMA

No. of Surgical Procedures	*Complications*					
	None		*Major**		*Minor†*	
	No.	*%*	*No.*	*%*	*No.*	*%*
0	16	100	0	0	0	0
1	21	72	3	10	5	17
2	4	67	0	0	2	34
3	0	0	2	50	2	50
4	0	0	1	100	0	0
5-9	1	33	2	67	0	0
≥10	2	50	1	25	1	25

*Major complications involved permanent cranial nerve injury.

†Minor complications included hematoma and seroma development, temporary cranial nerve weakness, and chyle leak.

TABLE 7. RELATIONSHIP BETWEEN AGE AT DIAGNOSIS AND PERSISTENCE OF TUMOR IN 31 PATIENTS WITH LYMPHANGIOMA

Age at Diagnosis	*Persistent Tumor*	
	No. of Patients	*%*
Birth*	14	45
<12 mo	5	16
1 to 3 y	5	16
>3 y	7	23

*Includes 1 patient with prenatal diagnosis.

Follow-up. The duration of follow-up in our patients ranged from 1.2 to 11.2 years. Of the 65 patients, 5 had been lost to follow-up. The 60 other patients or their parents were interviewed to determine whether the patients had received further treatment for the lesions, their current symptoms, and the current status of the lesion. Two patients had died of complications related to the lesion. One died at age 2 years, a month after operation at our institution. Death was attributed to swelling of the lesion and obstruction of the tracheostomy tube. This patient had an extensive lesion involving both sides of the neck, the oropharynx, the hypopharynx and/or larynx, and the infratemporal fossa. The second child (age 2) died 17 months after evaluation, but had received no treatment at our institution. This child also had an extensive lesion with involvement of the parotid gland, the posterior aspect of the neck, the parapharyngeal space, and the intratemporal fossa. He died of infection and swelling of the lesion. Both children had their lesion diagnosed at birth and had undergone treatment before our evaluation.

Of the 58 remaining patients, 31 (53%) thought that their lesion was still present. This was based on patient (or parent) observation and was not confirmed radiographically. The relationship between age of the patient at time of diagnosis and persistence or recurrence of the lesion is shown in Table 7. Overall, 23 (74%) of the 31 patients with persistent disease had complaints regarding their lesion. Since their evaluation, 16 patients (52%) reported that their lesion had gotten smaller, and 3 (10%) reported it was larger. Nine patients received further treatment after evaluation at our institution. Six underwent further surgical excision. One of these underwent debulking of an intraorbital lesion at our institution, and the other 5 patients underwent further surgical excision elsewhere. Three patients underwent laser debulking of their tumor, and 1 patient continues to receive interferon-alfa 2a on a regular basis. Two patients have undergone both surgical and laser therapy since their

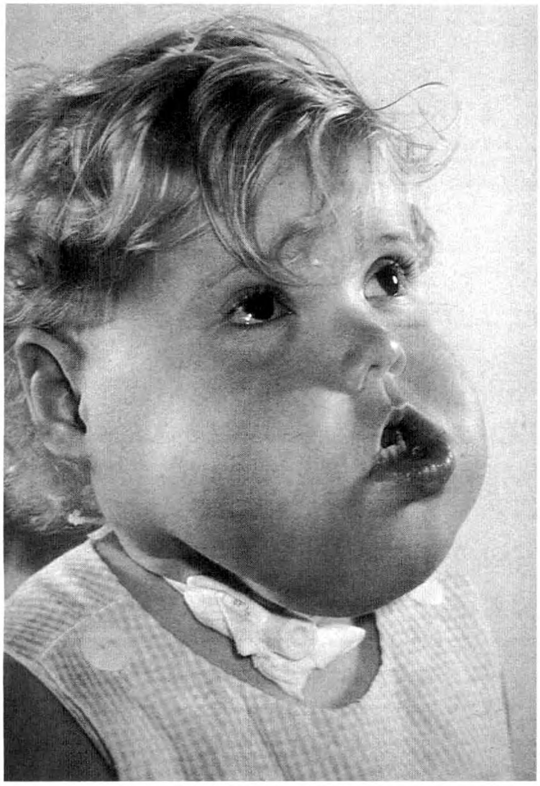

Fig 5. Patient with extensive lymphangioma throughout head and neck region.

evaluation at our institution.

DISCUSSION

The incidence of lymphangiomas has been reported[9] to be 1.2 to 2.8 per 1,000; thus, they are relatively uncommon pediatric lesions. Although most authors report that 50% or more are diagnosed at birth, only 35% of the lesions in our patients were diagnosed at birth, and 27% were diagnosed after the age of 3 years. Our near-equal distribution of male and female patients agrees with that in the literature.[10-12] None of our patients had a prenatal history that would have contributed to the development of the lesion. This finding also is in agreement with previous findings; most authors believe that cystic hygromas discovered in the first 2 trimesters of pregnancy represent an entity (with similar origins) separate from the lymphangiomas discovered at birth or in early childhood.[13,14] Cystic hygromas occurring early in gestation are associated with fetal hydrops and a high incidence of fetal death. In the 2 patients in our study who were stillborn, the diagnosis was made before the third trimester, and thus the lesions were not considered true lymphangiomas. Both of the fetuses also had multiple other congenital defects.

Although difficult to establish with certainty, the varied onset and character of cervical lymphangiomas noted on prenatal ultrasonography support multiple etiologic factors and a continuum with lesions noted at birth. Bronshtein et al[15] documented prognostic features of septated and nonseptated cystic hygromas noted early in the second trimester. Septated hygromas were associated with a 72% rate of dyskaryosis and a live birth rate of only 12%. Nonseptated cystic hygromas were strikingly different — the dyskaryosis rate was 5.7% and the live birth rate was 94%. The septations were suggested to represent complete jugular lymphatic obstruction, and nonseptated cystic hygromas to represent transient obstruction or delayed communication of the lymphatic sacs to the venous system. Alternatively, septated forms could result from lymphatic obstruction with subcutaneous edema, and nonseptated forms could simply be a delayed form of lymphatic accumulation noted in normal development. Regardless of the explanation, the association of chromosome abnormalities and structural abnormalities warrants consideration of karyotyping and close evaluation of patients with lymphangiomas at birth.

Histologically, these tumors are composed of dilated lymphatic channels with 1 or several endothelial layers with or without an adventitial layer. The dilated spaces can vary from microscopic to large and cystic. Grossly, they are ill defined and can be colorless to bluish. The most common presentation of these lesions is a soft, painless mass that may enlarge with time or in response to trauma[16,17] or to an upper respiratory tract infection.[11,17,18] Hemorrhage into the lesion also has been responsible for sudden enlargement. Unlike hemangiomas, lymphangiomas are not believed to involute spontaneously,[19] and most often, they increase in size as the child grows. The literature has scattered reports of spontaneous involution,[11,17] and 4 of our patients who received no treatment experienced apparent resolution of the lesion. Spontaneous resolution must be viewed skeptically because of the lack of histologic or radiologic data to confirm the diagnosis in these cases.

Most of our patients (88%) presented with a mass, and only 8 (12%) complained of any associated discomfort. All patients with intraorbital tumors experienced some degree of proptosis (10 of 10 patients with intraorbital involvement). Eight patients (12%) experienced respiratory distress. All but 1 of these patients had considerable involvement of the oral cavity, tongue, or hypopharynx and/or larynx, and 5 required tracheotomy. One of the patients with extensive neck, oral cavity, and upper airway involvement is shown in Fig 5. Other complaints related to the mass included dysphagia and dysphonia, which were related to the location of the lesion. In general, lesions involving structures in the upper aerodigestive tract were more likely to create symptoms.

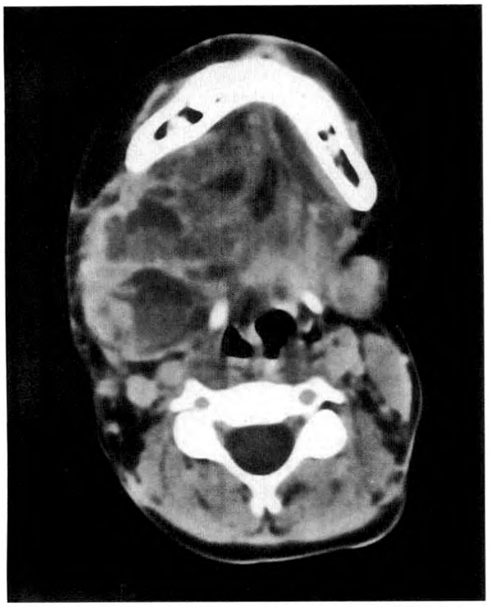

Fig 6. Computed tomogram of large lymphangioma of neck and oral cavity.

Diagnosis of these lesions was aided by both clinical presentation and radiologic evaluation. Only 11 patients did not receive any type of radiologic evaluation before treatment, in most cases because the lesion was so small or so superficial it was not believed necessary either to diagnose or to establish the extent of the tumor. Evaluation in 56% of our patients included computed tomography, which portrays lymphangiomas as low-density cystic lesions, most often multilocular with septa that vary from thick to thin (Fig 6). The usefulness of a contrast agent is unpredictable, because it provides enhancement in only 50% of cases.[20] Although not used as frequently, magnetic resonance imaging can help outline the extent of a large lymphangioma (Fig 7) and aid in planning a surgical approach. Best depicted on T2-weighted images, the mean signal intensity of lymphangiomas is greater than that of muscle, cerebrospinal fluid, and fat. Gadolinium provides no additional benefit in evaluating these lesions.[21] Ultrasonography also can be useful, especially in more superficial lesions, and can help detect recent hemorrhage.[22] Our patients underwent various radiologic evaluations, and twice as many underwent computed tomography (54%) as magnetic resonance imaging (23%) and ultrasonography (15%). The most likely reason that computed tomography was the imaging study most often used involves its ability to better delineate bony structures and thus indicate the extent of the lesion, as well as the clinician's comfort in interpreting these films when compared with magnetic resonance imaging or ultrasonography.

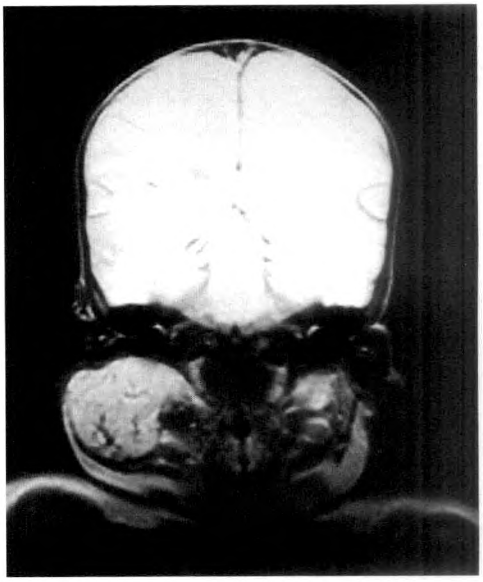

Fig 7. Magnetic resonance image of large lymphangioma.

The location of the lymphangioma is extremely important in determining the chance of complete excision, recurrence, and/or complications. Some studies have considered the posterior cervical area to be the most common location for these tumors.[9,10] In our series of patients, however, the submandibular area was the most common site (37%), and the parotid gland (31%) was the second most common site. Only 8 patients (12%) had involvement of the posterior area of the neck (Table 1). As would be expected, there was a high correlation between the number of locations involved with tumor and the number of surgical procedures required (Table 2). The location also influences the resectability of the tumor: single-site neck or parotid lesions were most likely to be resected in a single procedure and had a low rate of recurrence, whereas tumors involving the upper aerodigestive tract (Table 5) and extensive lesions involving multiple sites were unlikely to be completely and permanently excised. Other authors[11,23] have suggested that lesions involving the floor of the mouth and the tongue are associated with an increase in persistent disease. Our patients with involvement of the cheek, lip, and hypopharynx and/or larynx also had a high rate of recurrence or persistence (Table 5). Intraorbital and intracranial tumors are virtually impossible to resect completely and subsequently have a high rate of persistent tumor (Table 5). We also examined whether the more extensive development of the lymphatic system on the left side of the body could contribute to the location of these tumors. Because the distribution of these tumors toward left and right sides was virtually equal, the thoracic duct must not play an important role in the development of these lesions.

The results of this study and others[7,11] confirm the challenge in treating patients with involvement of

TABLE 8. PROPOSED STAGING FOR LYMPHANGIOMAS

Stage	*Features*
T0	No functional deficit
	No cosmetic alteration
	Single site of involvement
	Age >1 y at diagnosis
T1	No functional deficit
	Minor cosmetic change
	Single site of involvement
	Any age at diagnosis
T2	No functional deficit
	Minor cosmetic changes
	Two or more sites of involvement
	Any age at diagnosis
T3	No functional deficit
	Major cosmetic changes
	Three or more sites of involvement
	Age <1 y at diagnosis
T4	Functional deficit
	Major cosmetic changes
	Multiple sites of involvement
	Diagnosed at birth

the floor of the mouth and tongue. The patients in this study who had such involvement uniformly had persistent disease, and 35% of those with tongue involvement reported difficulty moving the tongue. Only 2 patients experienced hypoglossal nerve injury, and 9 others experienced limitation of tongue motion due to residual tumor or scarring and fibrosis. Our preference has been to maximally reduce the lymphangioma in the neck with nerve preservation and to minimize direct tongue reduction in hopes of maintaining tongue mobility.

An important consideration in the care of patients with lymphangiomas is the cosmetic consequences of the malformation and the concerns of the parents and child related to the mass effect. This element of managing lymphangiomas is not mentioned in prior reports. Factors that influence the cosmetic consequences include the proximity of the lymphangioma to the superficial subcutaneous tissues and the site of the malformation. Lesions involving the superficial subcutaneous tissues of the anterior aspect of the face and lips are extremely difficult to treat because of the size of the facial nerve branches, the difficulty in raising skin flaps in this anterior extent, and the infiltrative characteristics of the lesion, which will almost assuredly leave residual disease and fibrosis. Proximity to the craniofacial skeleton can alter bone growth and produce facial asymmetry, and finally, the operation can result in cranial nerve dysfunction with resultant cosmetic consequences.

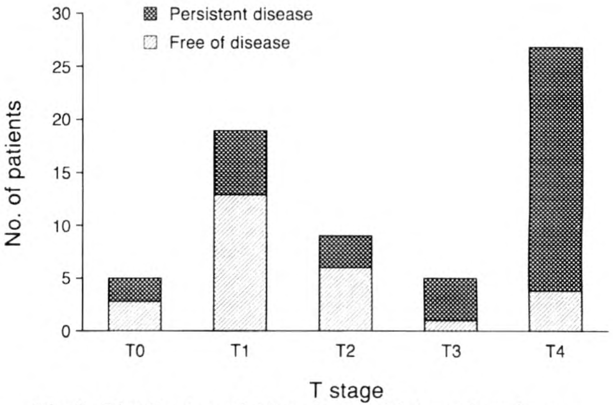

Fig 8. Distribution of 65 patients with lymphangiomas, by stage of disease.

The variables of functional impairment, cosmesis, number of locations, and age at diagnosis have been used to develop a staging system. In this system, functional impairment is defined as altered swallowing, speech, vision, or respiration and is the primary means of defining T4 lesions. Cosmetic changes are considered absent, minor, or major. Minor changes include mild facial asymmetry, whereas major changes include bony facial asymmetry and considerable involvement of the subcutaneous tissues of the lips and face. Site of involvement is defined by the sites listed in Table 1, and staging is based on the number of these sites involved with lymphangiomas. Age at diagnosis is divided into birth, younger than 1 year, older than 1 year, and any age. The proposed staging system is outlined in Table 8. For cases in which the variables do not exactly match those of a particular stage, a systematic method is applied. If a patient has a functional deficit, the stage is T4; if there is no functional impairment, cosmesis is then considered. Thus, a patient with a major cosmetic change and no functional deficit whose variables otherwise do not fit a specific stage is considered to have stage T3 disease.

The distribution by stage of disease in our 65 patients is shown in Fig 8, and the distribution by stage in the patients who underwent surgical treatment is shown in Fig 9. Both Figures show an increase in persistent disease with increasing T stage. The incidence of complications also increased with higher T stage in our patients. Of the 10 patients who experienced a major complication, 8 (80%) had T4 lesions (42% of the patients with T4 disease who had surgery), 1 had T3, and 1 had T2. Thus, the likelihood of experiencing a major complication increased with increasing T stage. Finally, the number of surgical procedures also increased with increasing T stage. Of the patients with T4 disease who had surgery, 63% underwent 2 or more procedures, whereas of the patients with T1 disease who had surgery, 86% of the patients underwent a single procedure.

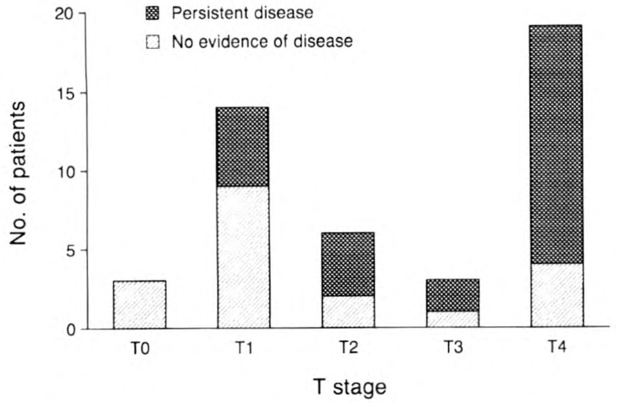

Fig 9. Distribution of 49 patients with lymphangiomas who underwent operation, by stage of disease.

Treatment for lymphangiomas remains primarily surgical. To date, the only means of complete removal is surgical excision, and it remains the therapy of choice for most patients.[10-12,18] The location of lesions within and surrounding vital structures and infiltrating the upper aerodigestive tract has led to a search for alternative treatments, especially for patients whose lesions are less amenable to surgical resection. Despite early success with irradiation for these lesions,[24,25] this approach largely has been abandoned because of the risk of malignancy developing later in life in these young patients. Other treatments have been directed at sclerosis of these primarily cystic lesions. Included in this category is the use of intralesional steroid therapy, which seems to provide minimal benefit,[26] and the use of chemotherapeutic agents, most often bleomycin sulfate. The routine use of bleomycin intralesional therapy, although thought to be helpful for decreasing the size of these lesions, virtually has been abandoned because of the risk of pulmonary fibrosis, an unacceptable risk in the treatment of a benign disease.[27,28] The most recent sclerosing therapy used for these lesions is OK-432, a biological preparation consisting of lyophilized powder containing cells of the *Streptococcus pyogenes* SU strain treated with benzylpenicillin potassium. It has been used in the past for treatment of malignancies in Japan, but its use in the United States has been limited. Several recent studies, primarily from Japan, have shown that intralesional use of this drug is effective for decreasing the size of lymphangiomas without obvious effects on the overlying skin.[8,29-32] None of our patients were treated with OK-432. Interferon alfa-2a has been used for treatment of similar lesions. The rationale for considering interferon alfa-2a therapy in patients with lymphangiomas relates to its effectiveness in treating hemangiomas.[33-35] The mixed nature of lymphangiomas prompted consideration of this treatment in masses presumed to have a considerable vascular component. Only 2 patients in our series received interferon as part of their treatment, and the benefit is debatable. Both patients had extensive tumors involving several areas of the head and neck region, and the interferon seems primarily to have helped to keep the lesion from progressing.

Recent advances in identification of a potential lymphatic endothelial growth factor receptor in developing lymphatic tissue, lymph nodes, and lymphangiomas should potentially foster the development of alternative treatment strategies.[36]

The treatment of choice, surgical therapy, is not without complications. The loss of cranial nerve function is a recognized risk in attempted resection of lymphangiomas, and it occurs at a high rate. The rate of permanent cranial nerve injury in our patients was 20%, and most often the facial nerve was affected. This finding is consistent with those of other reports.[10-12,37] Other systemic complications, including sepsis and thrombocytopenia, have been known to occur,[12] but they were not observed in the patients treated at our institution. Minor complications such as the development of hematomas and seromas postoperatively are even more common,[11,12] and they may require acute or serial drainage procedures. There is a trend toward an increase in complications with additional operation (Table 6), and this suggests an increased rate of morbidity in patients who do not have their disease eradicated at initial operation.

In general, patients with lymphangiomas tend to do well. Twenty-seven of our patients had no further evidence of tumor at follow-up, and an additional 8 patients with persistent tumor had no complaints. The recurrence rate of 39% for all patients who underwent operation at our institution is favorable when compared with the rate of 50% commonly reported in the literature.[11] For the 23 patients who underwent attempted total resection as their initial procedure at our institution, the recurrence rate was 21%, which is in agreement with those in the literature.[11,12] Because of the benign nature of these tumors, the risk of death should be low; however, mortality rates have been reported as high as 3.4% to 5.7%.[12] In patients with extensive tumors involving multiple areas, problems can occur, as evidenced by the 2 children (3%) in our series who died of complications related to their tumor. Lymphangiomas involving the upper aerodigestive tract can swell and, without proper airway protection, lead to respiratory distress or death. Even tracheotomy is not foolproof, as shown by the child who died of swelling of the lesion and subsequent obstruction of the tracheotomy tube. Infectious complications also can be devastating, as noted in the child who died of complications related to infection. It is important to remember that even though these lesions are benign, they can lead to serious complications.

CONCLUSIONS

1. Patients with lymphangioma diagnosed at birth should have karyotype analysis.

2. Age at diagnosis of lymphangioma is correlated with the likelihood of recurrence and the number of operations required.

3. A greater number of sites of involvement increases the probability of recurrence and the number of operations.

4. Cosmesis is a major consideration in the management of these lesions.

5. Lesions involving the lip, the hypopharynx and/or larynx, and the tongue and floor of the mouth have high rates of recurrent or persistent disease.

6. A staging system based on functional deficit, cosmetic changes, number of sites of involvement, and age at diagnosis is proposed for future use and modification.

7. Our treatment recommendations rely on careful and meticulous surgical excision as a primary approach, and because of the tendency for these lesions to grow and invade surrounding structures, early intervention is advocated. The use of a "watch-and-wait" attitude is discouraged.

8. Advances in the molecular biological characterization of these malformations may generate new treatment options.

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SLOW-RELEASE 5-FLUOROURACIL AND TRIAMCINOLONE REDUCES SUBGLOTTIC STENOSIS IN A RABBIT MODEL

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A previous pilot study suggested that a sustained-release conjugate that provided a slow release of 5-fluorouracil and triamcinolone acetonide injected into the tracheal and paratracheal tissues of rabbits at the time of subglottic surgery reduced the formation of subglottic stenosis. Our study was undertaken to confirm the effect. Ten milligrams of the compound suspended in hyaluronic acid was injected at the time of injury via a laryngofissure approach. The results showed that the control group had a mean stenosis of 52%, whereas the treated group had a mean stenosis of 32%. There was a significant difference between the treated and untreated groups ($p = .003$). It is hoped that this co-drug ultimately can be used in humans to reduce stenosis formation after laryngotracheal surgery and in other forms of otorhinolaryngological surgery.

KEY WORDS — 5-fluorouracil, subglottic stenosis, triamcinolone acetonide.

INTRODUCTION

Before the modern skills of neonatal resuscitation had been developed,[1] the majority of subglottic (or laryngotracheal) stenoses were congenital,[2] but since the 1960s as many as 92% of cases have been acquired.[3] Such stenoses may be caused by damage to the tissues of the larynx and trachea by traumatic, repeated, or prolonged intubation required for ventilatory support. Other causes may include infection, inhalation of hot or toxic substances, and local or systemic inflammatory diseases.

Treatment of subglottic stenosis is varied and depends on the severity of the stenosis and the symptoms produced.[4] Mild cases may improve spontaneously as the patient's airway grows and matures, whereas moderate stenoses may be treated by endoscopic procedures.[5] Endoscopic treatment is indicated only if the stenosis does not involve scarring wider than 1 cm vertically. Circumferential scarring can only be treated in this way if the cartilage framework remains intact. In addition, there must be no associated tracheomalacia, cartilage loss, or chronic infection. An indwelling tracheotomy is therefore a poor prognostic feature because of the inevitable chronic low-grade infection.[6] Open operations that involve splitting the cricoid anteriorly and stenting may be needed for neonates who cannot be extubated because of incipient stenosis.[7] Cartilage grafts placed anteriorly[8] or posteriorly[9] may be needed to maintain airway patency in more severe cases of mature stenosis or when endoscopic treatments fail.

It is important to minimize the surgical damage to the airway to reduce new scar formation and contraction. However, there is a risk of restenosis with every form of treatment, whether from continuation of the initial chronic inflammatory process or as a result of the surgical injury. A variety of topical and systemic medical treatments have been used in the past to reduce the rate of stenosis formation, but their use has not been shown to be helpful.[10]

A new drug delivery system, a slow-release compound (5FU/TA) that delivers 5-fluorouracil (5FU) and triamcinolone acetonide (TA), has been investigated to determine whether it would reduce the amount of scar formation in extraocular muscle surgery.[11] 5-Fluorouracil is known to have an antiproliferative action against cultured fibroblasts, but it is not cytocidal at concentrations used in ophthalmic surgery.[12] A pilot study was undertaken, using a rabbit model, to determine whether this co-drug would

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From the Royal Berkshire Hospital, Reading, England (Ingrams), and the Otolaryngology Research Center (Ingrams, Dhingra, Shah, Shapshay) and the New England Eye Center (Ashton), New England Medical Center, Boston, Massachusetts. Supported by National Institutes of Health/National Eye Institute grant EY 11041 and by Control Delivery Systems, Inc, Watertown, Massachusetts (P.A.). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Tufts University School of Medicine, New England Medical Center.

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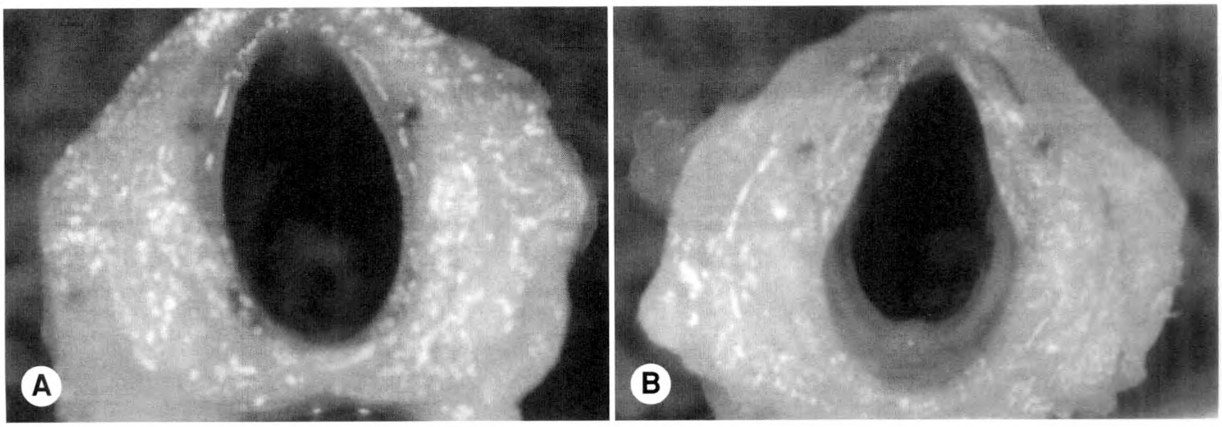


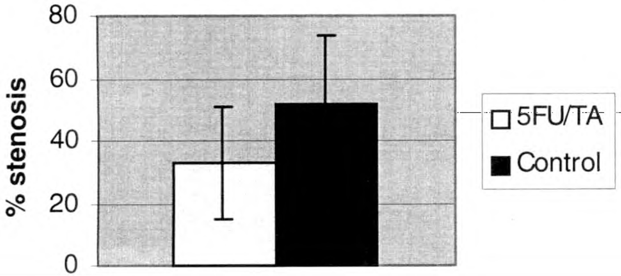
Fig 1. Larynges sectioned at subglottis. A) Treated larynx. B) Control larynx.

reduce the amount of stenosis in the subglottic area after a standardized injury. The untreated control group had a mean stenosis of 47% at 12 weeks, compared with a mean of 15% in the 2 treated groups combined.[13] The groups were too small for meaningful statistical analysis, but the results were encouraging, and so this present prospective randomized controlled study was set up to investigate this effect. The drug had been used in 2.5-mg and 12.5-mg amounts in the pilot study, but no dose effect was clear, and it was decided that a single dose of 10 mg would be used for this study.

METHOD

The technique used was as previously described in the literature.[13] We assumed a null hypothesis that the rate of formation of stenosis in the rabbit airway after curetting was the same in animals treated with 5FU/TA and in untreated animals, and an effect size (based on the pilot study) of approximately 20%. If α (maximum chance of incorrectly rejecting the null hypothesis) is taken as 0.05 and β (chance of missing an association and incorrectly accepting the null hypothesis; $1-\beta$ = power of test) as 0.10, and a 1-tailed test is used, standard sample size tables suggest that approximately 20 subjects would be needed in each group to achieve statistical significance.[14] Thirty-nine 3- to 4-kg single pathogen–free New Zealand White rabbits had their subglottic epithelium, submucosa, and perichondrium damaged in a standardized fashion, via a laryngofissure approach. One other animal died during operation. Lateral bands of mucosa were left intact, because previous experience had shown that circumferential injury resulted in fatal tracheal edema.[13] Intramuscular xylazine hydrochloride and ketamine hydrochloride were used to provide general anesthesia.

Twenty randomly chosen animals were injected with 2.5 mg of the co-drug suspended in 0.2 mL of hyaluronic acid gel. The material was injected into tissues adjacent to the injury. Nineteen control animals were injected with 0.2 mL of hyaluronic acid gel alone. All animals in both groups were painlessly killed after 3 months. The larynx and proximal trachea were removed from each animal and immediately fixed in 10% formalin solution. The specimens were sectioned transversely at the level of the superior border of the cricoid cartilage and photographed with a 2:1 macro lens at a fixed distance. Measurements of area were made with standard computer morphometric software at the level of sectioning and at the level of any stenosis. The stenosis was expressed as the percentage of the airway at the level of the superior border of the cricoid (which we assumed was a good approximation of the original mid-cricoid airway) that was occupied by stenotic tissue. This method was the same as previously described.[13] All results were obtained blind.



RESULTS

As in the pilot study, all wounds healed without infection, and there were no cases of tracheocutaneous fistula or subcutaneous emphysema. There were no symptoms or signs of local or systemic toxicity. The treated group had a mean stenosis of 31.7% (SD, 18.3; Fig 1A), and the control group had a mean

[redacted]

DISCUSSION

The results of our study confirm the impression gained in the earlier pilot study that the 5FU/TA co-drug can be used in this small animal model to reduce the amount of stenosis formed by a standardized injury. The co-drug has been used successfully in ophthalmology to reduce scar formation in extraocular muscle surgery and to inhibit epithelial down-growth and proliferative retinopathy.[11] The co-drug is designed to release a very small amount of active 5FU and TA over a long time. A labile carbonate bond that makes them inactive and largely insoluble in water combines the drugs. The resulting compound is stable in suspension, but once it is in solution, rapid hydrolysis of the carbonate bond occurs to regenerate active 5-FU and TA. Release of the active drugs thus occurs as the co-drug slowly dissolves over weeks or months. Because of the extremely low solubility of the co-drug and its rapid hydrolysis once in solution, no intact co-drug can be detected in solution.[15] Because the concentration of the active drugs always remains extremely low, the risk of toxicity is reduced, and no evidence of systemic toxicity was seen in this study or the earlier pilot study.[13] Histologic examination was not repeated in this study, but the pilot study showed that at 1 week there was persistence of ulceration in treated and control groups, with similar amounts of inflammation in each; at 2 weeks all wounds had healed, with a reduction in the amount of inflammation when compared with the 1-week group; and at 12 weeks all wounds had healed, with increased chronic inflammation and fibrosis formation in the control group as compared with the treated group. There was no evidence of cartilage resorption.[13]

It is hoped that further research with this material will show its use in reducing restenosis in patients who undergo surgery to relieve subglottic stenosis, and it is possible that it will also be of use in other aspects of otorhinolaryngological surgery, such as sinus surgery and dacryocystorhinostomy.

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ENDOSCOPIC REVISION SURGERY FOR RECURRENT EPIPHORA OCCURRING AFTER EXTERNAL DACRYOCYSTORHINOSTOMY

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Recurrent epiphora after external dacryocystorhinostomy (DCR) can be a frustrating experience for both the patient and the physician. We present the results of a retrospective study of 18 patients with epiphora recurring after external DCR who were operated upon to recreate lacrimal drainage through an endoscopic endonasal approach. The procedure was successful in 83.3% of cases, with no major complications. The results are comparable to those of an external approach, but the cosmetic advantages are clear. It is a 1-stage procedure that permits correction of associated intranasal disease that may be a causative factor in the recurrence of lacrimal obstruction. The endoscopic endonasal approach is a relatively safe and reliable option in the management of recurrent epiphora.

KEY WORDS — dacryocystorhinostomy, endonasal surgery, lacrimal obstruction, nasal endoscopy, nasolacrimal duct.

INTRODUCTION

Dacryocystorhinostomy (DCR) is indicated in patients with chronic stenosis of the nasolacrimal duct (postsaccal stenosis) when manifestations such as epiphora or repeated infections are severe enough to be annoying.[1] External DCR was first described by Toti[2] in 1904 and was modified in 1921 by Dupuy-Dutemps and Bourguet,[3] who directly sutured the cut edges of the nasal and lacrimal sac mucosal flaps. In 1962, Jones[4] added bicanalicular silicone tubes. Endonasal DCR was first introduced by West[5] in 1911 to avoid a skin incision, but it was inherently limited by poor visibility within the narrow confines of the superior nasal cavity. Resurgence of interest in endonasal techniques came with the advent of the rigid nasal endoscope with angled fields of view, which provided excellent intranasal visualization. A cadaver study demonstrating the feasibility of endoscopic endonasal DCR was first performed by Rice[6] in 1988, but the first clinical study was published by McDonogh and Meiring[7] in 1989. Massaro et al,[8] in 1990, pioneered the use of nasal-to-lacrimal lasers for the creation of the DCR fistula. Initially, that group used the argon laser, but they changed to carbon dioxide and potassium titanyl phosphate–yttrium-aluminum-garnet lasers in a subsequent clinical study.[9] Woog et al[10] adapted the holmium–yttrium-aluminum-garnet laser to this procedure. Christenbury[11] in 1992 described his experience in forming a lacrimal-to-nasal fistula by delivering argon laser energy through either canaliculus. Pearlman et al[12] used a neodymium:yttrium-aluminum-garnet laser in the same direction.

External DCR, with its established 90% or greater success rate, has remained the gold standard by which all newer methods of DCR must be judged.[8] However, recurrent epiphora after external DCR can be a frustrating experience for both the patient and the physician. The surgeon is faced with the dilemma of whether to reoperate. Revision external DCR typically requires reopening of the skin incision in an attempt to reestablish lacrimal drainage through an already scarred surgical field.[13] Very often, ophthalmologists tend to treat recurrent epiphora by medical means for extended periods before pursuing a surgical solution. It is for these reasons that we present the results of a retrospective study of 18 patients with epiphora recurring after external DCR on whom we operated to recreate lacrimal drainage through an endoscopic endonasal approach at the Tanta University Hospitals, Tanta, Egypt, over the 3-year period from 1994 to 1996.

PATIENTS AND METHODS

Between January 1994 and November 1996, 21 patients underwent 21 endoscopic endonasal revision DCRs. All procedures were performed by a joint team including the same otolaryngologist (A.E.G.) and 1 of 2 referring ophthalmologists (A.D., M.G.). The indication for surgery was recurrent epiphora after failed external DCR. The length of time between the primary external DCR and the revision endoscopic DCR ranged from 9 months to 5 years. The diagnosis necessitating primary DCR was distal lacrimal obstruction with dacryocystitis. Nasolacrimal duct obstruction was diagnosed by lacrimal probing and irrigation studies, by Jones primary and secondary dye tests, and by dacryocystography in some patients. The

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techniques used for the primary external DCRs varied among referring surgeons; ophthalmologists used curettes and microrongeurs for bone removal and stented the lacrimal fistula by bicanalicular silicone tubing, whereas in cases done by otolaryngologists, bone was removed by a drill and the fistula was not intubated. In all cases, lacrimal sac and nasal mucosal flaps were sutured.

The patient was placed in a supine position with the head of the operating table elevated approximately 15° to reduce venous pressure at the operative site. The procedure was performed under local anesthesia with neuroleptanalgesia. The patient was draped with the nose and affected eye in the surgical field. The nasal cavity was vasoconstricted with cottonoids soaked in equal parts of 0.05% oxymetazoline hydrochloride and 4% lidocaine hydrochloride, placed along the lateral nasal wall and middle meatus. Then, the lacrimal fossa and lateral nasal wall were infiltrated with 1% lidocaine hydrochloride and 1:100,000 epinephrine hydrochloride.

The operative site was then inspected with a rigid nasal endoscope 4.0 mm in external diameter and with a 30° viewing angle. The endoscope was attached to a video camera; this arrangement enabled the assisting ophthalmologist to observe all endonasal manipulations on a video monitor. The anterior end of the middle turbinate was trimmed if it was enlarged enough to hide the operative site, or if it had been the source of prior adhesions. If septal deviation was so severe that adequate visualization and access to the lateral nasal wall was blocked, septoplasty was considered necessary. A straight septum also allowed for easier examination and cleaning of the surgical site after surgery.[14] Ethmoidectomy was done in the presence of polypoidal disease or infected agger nasi cells.

The lacrimal sac was located along the superior aspect of the lateral nasal wall, just anterior to the middle turbinate. External finger pressure, a lacrimal probe passed through a canaliculus medially, or shining a cold light from the outside over the lacrimal fossa can also help identify the sac in difficult cases. The lacrimal fistula was then inspected for size, site, and surrounding tissue relationships. Very often, the fistula was found closed by fibrosis, granulation tissue, or adhesions. Removal of granulation tissue or lysis of adhesions with or without removal of the anterior end of the middle turbinate may be needed to reestablish the patency of the lacrimal fistula. In case of tight stenosis around the silicone tube or complete closure of the ostium after the tube was removed, reopening of the sac was necessary. A 00 Bowman's lacrimal probe introduced through the upper canaliculus was advanced medially to tent the mucosa of the lateral nasal wall. A sickle knife was used to make a vertical incision anterior to the underlying probe tip. A straight Blakesley forceps was employed to remove 1.0 to 1.5 cm^2 of nasal mucosa over the probe tip. Frequently, there was additional scar tissue that needed to be removed with an angled Blakesley forceps to enter the sac interior. The entire medial wall of the sac was removed; the surgeon removed only tissues surrounding the probe. Visualization directly into the sac can often identify the internal common punctum. Enlargement of the fistula included the posteroinferior portion of the sac immediately superior to the obstructed nasolacrimal duct. Bicanalicular silicone tubing was used only in exceptional circumstances, mostly in cases of severe fibrosis of the sac, and removed 2 months later. No nasal packing was used, unless bleeding was a problem or septoplasty had been performed. Lacrimal irrigation was carried out at the end of the operation and on the first postoperative day.

The patient was discharged on the day of surgery or 1 day after. Postoperative medications included oral cephalexin, antibiotic-steroid eye drops, and saline nasal spray. Patients were not allowed to blow their noses during the first week after surgery. Patients were seen weekly for 2 months and then monthly for at least 1 year. Three were lost to follow-up and excluded from the study. Fistula patency was documented at each visit by the absence of symptoms, lacrimal irrigation, and demonstration of fluorescein flow from the eye to the nasal cavity by blue-light endoscopy. Crusts and debris about the nasal ostium were gently cleared, and early granulations were trimmed or cauterized until the wound had stabilized.

For each patient, records on demographic data, history, physical examination findings, lacrimal patency test results, prior surgical procedures, operative findings, intraoperative and postoperative complications, and the fate of the fistula were stored in a computer database and analyzed.

RESULTS

This study included 18 patients with the diagnosis of recurrent epiphora after failed external DCR. Lacrimal bone was removed by a curette and a rongeur in 12 primary DCRs and by a drill in 6. Silicone intubation was used in 12 patients and removed 6 to 10 months after primary operation. The length of time between the primary and revision procedures ranged from 9 months to 5 years. The patients were 13 women and 5 men; their ages ranged from 34 to 57 years, with an average of 49 years. All procedures were uni-

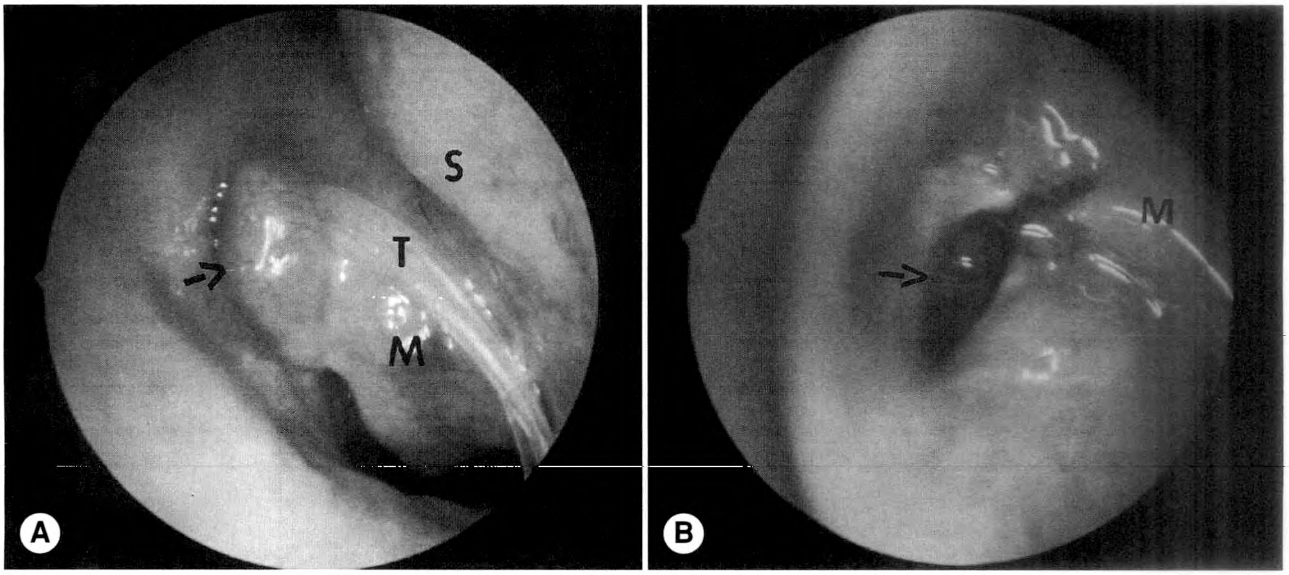

Fig 1. (Case 1) Endoscopic pictures of right nasal wall. A) Showing granulation tissue (arrow) at dacryocystorhinostomy fistula that obstructs tear flow. T — silicone tube, S — septum, M — middle turbinate. B) Showing wide dacryocystorhinos-

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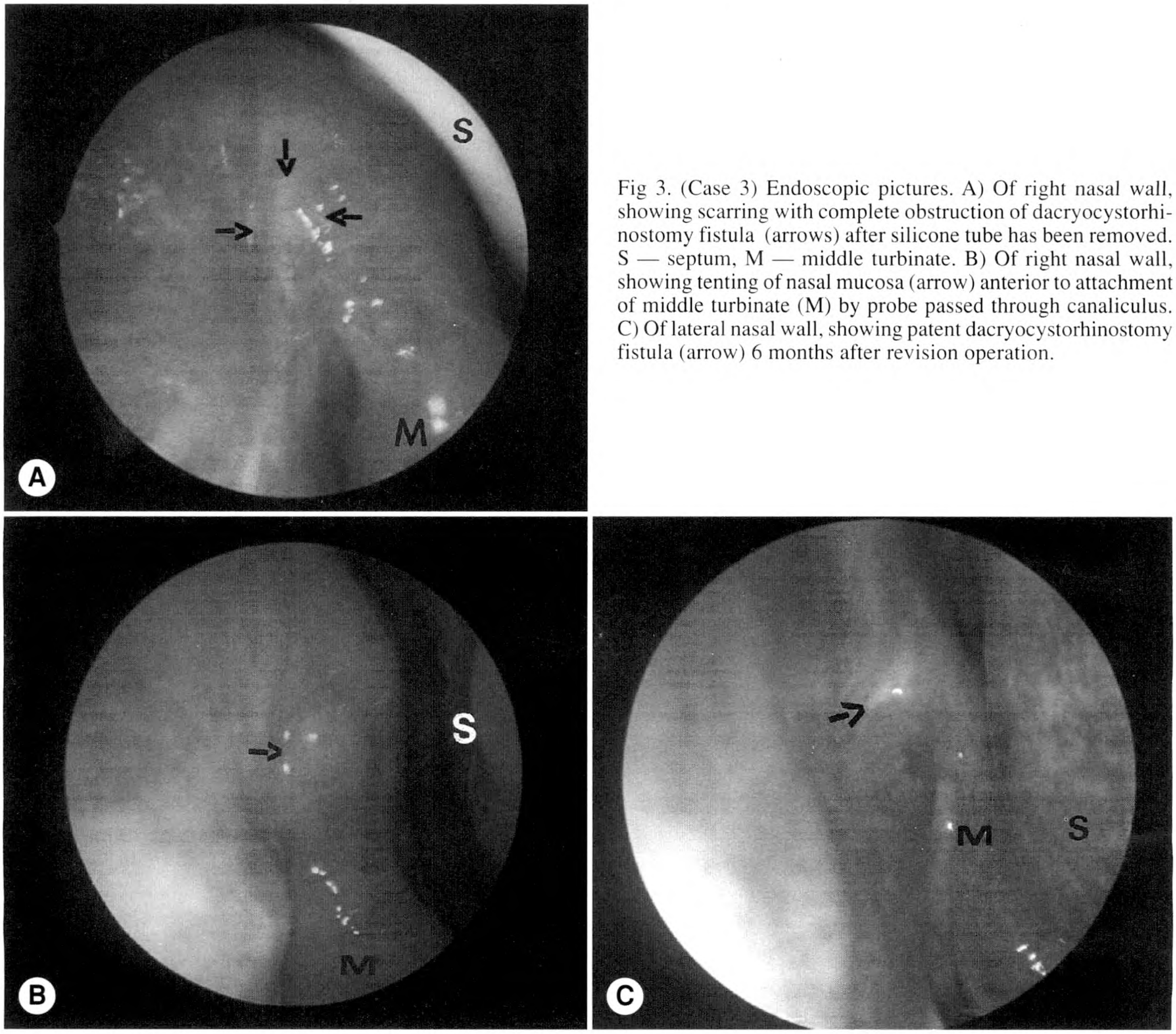

Fig 3. (Case 3) Endoscopic pictures. A) Of right nasal wall, showing scarring with complete obstruction of dacryocystorhinostomy fistula (arrows) after silicone tube has been removed. S — septum, M — middle turbinate. B) Of right nasal wall, showing tenting of nasal mucosa (arrow) anterior to attachment of middle turbinate (M) by probe passed through canaliculus. C) Of lateral nasal wall, showing patent dacryocystorhinostomy fistula (arrow) 6 months after revision operation.

agulated endoscopically with an insulated suction tip. There were no cases of diplopia, blindness, orbital hematoma, or orbital fat exposure.

The postoperative complications, other than recurrent epiphora, were 1 case of surgical emphysema and another case of synechia between the middle turbinate and the septum. Prolapse of the silicone tube at the medial canthus occurred in 1 case; this was treated by endoscopic repositioning of the stent. Fifteen patients (with 15 procedures) maintained a patent lacrimal fistula; ostium patency was correlated with symptomatic improvement in all cases. The fistula was closed by scarring and fibrosis in 3 procedures, with recurrence of epiphora. These 3 patients were scheduled for external revision DCR.

Endoscopic surgery is primarily a 1-handed procedure. Initially, this 1-handedness may appear as a handicap, but experience did not show this to be a problem. One may encounter some difficulty in avoiding contact between the lens of the endoscope and blood. Such contact required frequent withdrawal, cleaning, and reintroduction of the endoscope. As with all endonasal procedures, meticulous hemostasis is mandatory.

The key element in revision DCR is the precise identification of the lacrimal sac while the surgeon is viewing from the nose; he or she can then accurately resect just those tissues overlying the lumen, while avoiding damage to surrounding orbital and nasal tissues. We found that the 00 Bowman's lacrimal probe, as a marker within the sac, provides this key element of control.

DISCUSSION

Despite the high success rate of external
(90% or more), several disadvantages have be
ported: 1) cutaneous scar, 2) potential injury
cent medial canthal structures with interfe

the pump mechanism of the lacrimal sac, 3) bleeding during operation, and 4) longer hospital stay. To obviate these disadvantages, DCR has been performed by an intranasal approach. The use of the rigid nasal endoscope and endoscopic sinus instrumentation in performing primary and revision DCR has been well demonstrated.[13,15] Although the nasal endoscope provides excellent visualization of the surgical site, primary endonasal DCR may be technically challenging because of difficulties in bone removal with conventional endoscopic sinus instruments alone.[10] There is currently no bone-removing instrument specifically designed for this location.[15] The use of a laser or drill to facilitate bone removal during this procedure is associated with thermal damage to bone and surrounding mucosa that increases postoperative scarring and reduces the patency rate.[1,10,12] Use of cutting instruments, rather than burs or lasers, would reduce the degree of burning; however, these are as yet unavailable. The endonasal approach has thus been particularly useful in our experience in the management of recurrent epiphora after failed external DCR, in which the lacrimal bony wall has been resected.[16]

We continue to favor an external approach for primary DCR, because it offers unsurpassed exposure of the lacrimal drainage anatomy that allows intraoperative inspection for suspected or unsuspected abnormalities, permits biopsies to be performed relatively easily, and aids in suturing of adjacent flaps of lacrimal and nasal mucosa to provide a patent epithelial-lined tract for tear drainage. The cutaneous scar from external DCR infrequently causes a persistent cosmetic blemish; placing the incision in conformance with relaxed skin-tension lines may minimize the risk of a noticeable scar.[17] Serious bleeding during operation is unusual in patients with normal clotting function; careful dissection should allow comparable hemostasis for laser and external procedures.[18] Although laser-assisted endonasal DCR is an outpatient procedure, the use of laser, video-endoscopy, and silicone tubing increases the costs of the operation. Moreover, the long-term success rate of endonasal procedures has not reached the level of that of external procedures.[19] In our opinion, the role of the rigid nasal endoscope in primary DCR is limited to control of the nasal (inner) surgical field when the external route is being used, and to postopera- ve follow-up of the ostium until the wound has sta- ized.

ft tissue, rather than bony, occlusion of the sur- ostium has been shown to be the most frequent f failure of external DCR, as confirmed by obing, diagnostic endoscopy, and computed tomography.[20] The most common identified obstruction results from granulation tissue, fibrosis, and synechia between the opening and adjacent middle turbinate mucosa.[21,22] For revision DCR especially, the endoscopic approach is superior to the external approach.[1] The angled instruments developed for endoscopic sinus surgery allow the occluded ostium to be relatively safely reopened under direct endoscopic visualization, so that the ostium can be sufficiently enlarged and properly positioned. Placement of the ostium too close to the middle turbinate results in subsequent adhesion and occlusion[13]; placement of the ostium too high results in "lacrimal sump" syndrome.[23] Granulations can be easily trimmed and cauterized,[21] and adhesions are cut together with resection of the anterior end of the middle turbinate. The endonasal approach is a 1-stage procedure that permits correction of associated nasal disorders, such as septal deviation, middle turbinate hypertrophy, or polypoidal disease, that may be a causative factor in the failure of DCR.[1] It avoids a skin incision in an already scarred surgical field. It is a minimally invasive procedure, done under local anesthesia, with fast return of patients to normal activities, because surgical intervention limited to the actual fistula site decreases operative and postoperative morbidity and permits routine outpatient management with improved cost-effectiveness.[8] No part of the endonasal procedure precludes an external approach should revision surgery be unsuccessful.[21]

Our results (success rate of 83.3%) are commensurate with those in the literature. Mickelson et al[24] reported a 60% success rate for revision cases. Mannor and Millman[25] reported an 80% success rate. Önerci et al[26] reported successful outcome of revision endoscopic surgery in 3 of 4 patients. Metson[14] reported a success rate of 75%. Welham and Henderson[27] reported an 83% success rate. Boush et al[28] reported success in 5 of 6 cases (83.3%). Lacrimal sac cicatrization and intranasal synechia formation are the most important prognostic factors for revision surgery.[25] There is some evidence that silicone tubing increases the incidence of DCR failure by inciting a granulomatous reaction at the internal ostium, with subsequent stenosis.[29,30] The use of silicone stents was restricted in our study to those cases with otherwise narrow or traumatized lacrimal passages; in these cases, we recommend early removal of the tube (at 2 months).

CONCLUSION

The endonasal endoscopic approach has been shown to be a relatively safe, reliable option in the management of recurrent epiphora after failed external DCR.

The results are comparable to those of the external approach, but the cosmetic advantages are clear. Moreover, it is a 1-stage procedure that permits correction of associated intranasal disease that may be a causative factor in the recurrence of lacrimal obstruction.

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CLINICAL NOTE

FOURTH BRANCHIAL CYST PRESENTING WITH NEONATAL RESPIRATORY DISTRESS

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Fourth branchial cysts are quite rare. A neonate with a left lateral neck mass and respiratory distress was found to have a fourth branchial cyst, which was diagnosed with computed tomography and endoscopy. The characteristic computed tomography findings included an air-containing neck cyst, which was located at the anteromedial site of the common carotid artery with mediastinal extension. Endoscopic examination revealed an internal opening at the apex of the pyriform sinus, communicating with the cyst. Total excision of the cyst was performed, and the specimen, which showed ciliated columnar epithelium with a subepithelial lymphoid infiltrate, thyroid follicles, and thymic tissue, histologically confirmed the diagnosis.

KEY WORDS — branchial anomaly, fourth branchial cyst.

INTRODUCTION

Branchial anomalies are not-uncommon causes of neck masses encountered by otolaryngologists. Depending on their anatomic location, branchial anomalies are classified into first, second, third, and fourth anomalies. It has been estimated that 95% of branchial anomalies are derived from the second branchial arch, pouch, and cleft.[1] The majority of the remaining 5% are from the first and third branchial remnants. Fourth branchial anomalies are exceedingly rare, with less than 45 cases reported in the literature.[2] Branchial anomalies may occur in the form of a cyst, sinus, or fistula. A cyst can occur alone or in association with a sinus or fistula. A sinus is a blindly ending pouch with a single opening, either external or internal. The term fistula is applied to the anomaly when both external and internal openings are present.[3] The clinical presentation of a fourth branchial cyst may be a recurrent neck abscess, recurrent suppurative thyroiditis, or an asymptomatic neck mass.[4,5] In this report, we describe a case of a fourth branchial cyst presenting at birth with respiratory distress.

CASE REPORT

A full-term, 2.9-kg male infant was referred to the National Cheng Kung University Hospital at 11 days of age with neonatal respiratory distress. He was born after a normal pregnancy to a healthy mother by spontaneous vaginal delivery. Shortly after delivery, he became dusky and tachypneic and required supplemental oxygen. Because of worsening respiratory distress, he was transferred to our institution.

Physical examination revealed a soft, fluctuant mass in the left side of the neck, extending from the angle of the mandible to the clavicle. Tachypnea, respiratory stridor, and chest wall retraction were noticed. Arterial blood gas analysis revealed respiratory acidosis (P_{CO2} = 59.7 mm Hg, pH = 7.25, P_{O2} = 48 mm Hg, base excess = −1.4, under oxygen hood). The infant was incubated but not mechanically ventilated. A computed tomography (CT) scan (Fig 1) of the neck and chest showed a peripherally enhanced cyst measuring 7 × 5.4 × 5 cm in the retropharyngeal space and left side of the neck with an air-fluid level within it. The cyst extended caudally between the thyroid gland and the common carotid artery into the anterosuperior mediastinum.

Direct laryngopharyngoscopic examination (Fig 2) demonstrated an opening at the apex of the left pyriform sinus. Palpation of the patient's neck triggered copious secretion gushing out from the opening, confirming the communication of the opening with the cyst. Neck exploration was carried out at 16 days of age through a left horizontal cervical incision. A subplatysmal dissection was performed tracing the cyst deep to the clavicle and into the anterosuperior mediastinum, where the cyst ended blindly. Dissection was carried out through the thyroid gland, and a portion of the gland was removed in continuity with the cyst. The entire cyst was explored and led toward the pyriform sinus. Here it tightly adhered to the thyropharyngeal muscle. No attempt was made

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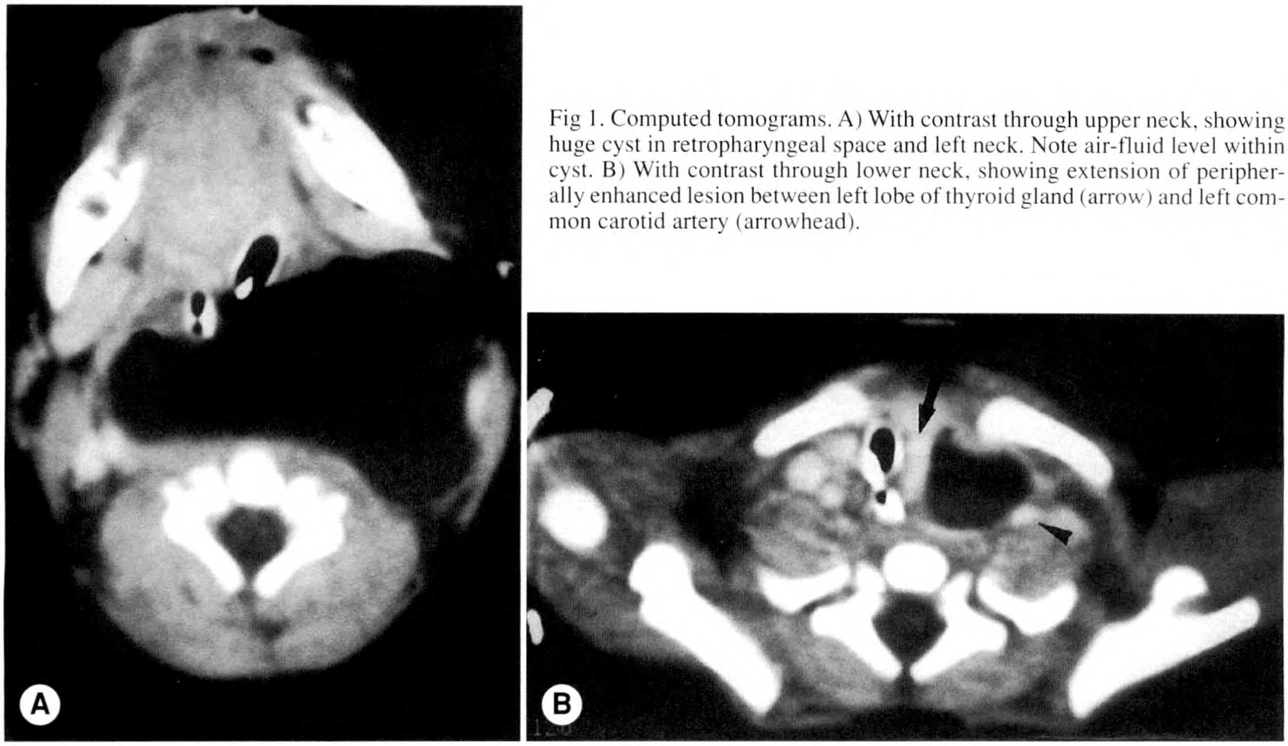

Fig 1. Computed tomograms. A) With contrast through upper neck, showing huge cyst in retropharyngeal space and left neck. Note air-fluid level within cyst. B) With contrast through lower neck, showing extension of peripherally enhanced lesion between left lobe of thyroid gland (arrow) and left common carotid artery (arrowhead).

to dissect out the sinus tract entering the pyriform sinus. The cyst was ligated and transected just lateral to the thyropharyngeal muscle. The patient had an unremarkable postoperative course and has been free of recurrence in 14 months of follow-up.

Histologic examination (Fig 3) showed a cystic lesion lined by ciliated columnar epithelium with subepithelial lymphoid infiltrate. Thyroid follicles and thymic tissue were noticed in the cyst wall.

DISCUSSION

Unlike the well-described pathways of first and second branchial anomalies, there has been controversy as to differentiation between those of third and fourth branchial anomalies.[3,6,7] It is presumed that a third branchial fistula arises from the base of the pyriform sinus anterior to the fold formed by the internal laryngeal nerve and courses rostral to the superior laryngeal nerve. After passing over the hypoglossal nerve, the fistula descends lateral to the carotid vessels and turns superficially to an external opening low in the neck. The theoretical pathway of a fourth branchial fistula begins at the apex of the pyriform sinus and descends adjacent to the thyroid gland, between the trachea and the common carotid artery, into the mediastinum. The fistula then ascends in the neck, posterior to the common carotid artery. After looping over the hypoglossal nerve, the fistula descends again to an external opening low in the neck.[8] Because of its long and tortuous pathway, a complete fourth branchial fistula has never been reported.

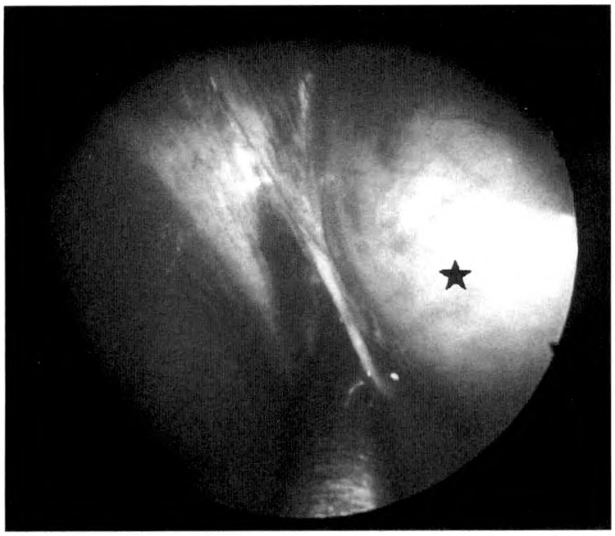

Fig 2. Direct laryngopharyngoscopic view of left pyriform sinus. Opening at apex is probed by suction tip. Star — left arytenoid cartilage.

The close proximity of third and fourth branchial anomalies makes them difficult to distinguish. They could be identified only by the site of internal opening and the relationships between the lesion and the carotid artery, nerves, thymus, thyroid gland, and parathyroid gland.[9-11] The anatomic evidence for fourth branchial origin of the present case was the site of internal opening at the apex of the pyriform sinus, the pathway anteromedial to the common c rotid artery, and the mediastinal extension of the c

Imaging evaluation of branchial anomalies include a barium swallow study, which may re

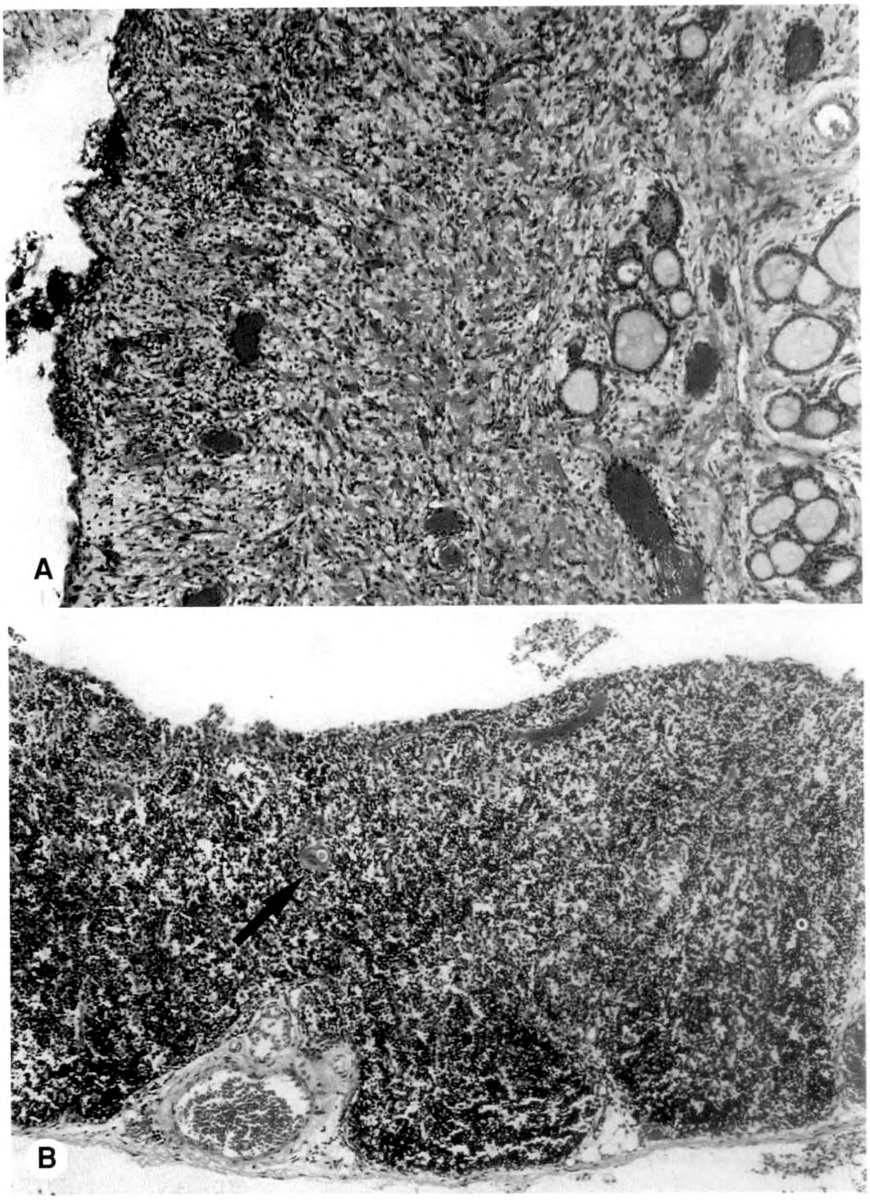

Fig 3. Histologic photomicrographs (H & E, original ×100). A) Cyst lined with ciliated columnar epithelium and subepithelial lymphoid infiltrate. Note thyroid follicles on right. B) Small lymphocytes (thymocytes) intermingled with epithelial cells and Hassall's corpuscles (arrow), characteristic of thymic tissue.

sinus tract extending from the pyriform sinus.[4,12,13] Applying sonography in cases presenting as acute thyroiditis is particularly useful. It reveals a characteristic perithyroid hypoechoic area that involves the left lobe of the thyroid gland.[14] A CT scan is the technique of choice for displaying both the location and extent of a branchial cyst, and delineating its infectious process. An air-containing lateral neck cyst represents the typical CT appearance of a branchial cyst.[15,16] This finding is very helpful in differentiating a branchial cyst from other cystic lesions of the neck, such as cystic hygroma, thyroglossal duct cyst, plunging ranula, and cervical thymic cyst. A CT scan may also contribute by outlining a sinus tract after njection of iodinated contrast media into the tract.[10] ome authors have adopted air as a contrast agent in T scanning for the same purpose.[17]

ndoscopy is the most effective means of direct visualization of the internal opening of the sinus tract. Instillation of methylene blue or catheterization with a Fogarty catheter during endoscopy is also advocated to define the sinus tract.[5,18,19] An internal opening has been rarely described in neonates.[3,18] We presented a neonate in whom the communication of the pyriform sinus and the cyst was well demonstrated by endoscopy.

Histologic examination will sometimes give indirect evidence of the origin of branchial anomalies. Ectopic thymic tissue in conjunction with branchial cyst has been a controversial issue in the literature. Because the thymus principally originates from the third branchial pouch, a branchial cyst with thymic tissue must be of third branchial origin.[4] However, some thymic tissue may develop from the ventral wing of the fourth branchial pouch; therefore, a fourth branchial cyst can also be associated with thymic tissue.[14,18] Because the fourth branchial pouch contributes to the formation of the thyroid gland, the presence of thyroid tissue suggests a fourth branchial origin.[18] The coexistence of thyroid and thymic tissues in the cyst wall further confirmed the fourth branchial origin in the present case, in addition to the anatomic evidence.

Fourth branchial cysts are rare, and the diagnosis should be considered in any patient with a history of recurrent lower neck abscess or recurrent suppurative thyroiditis, or in any neonate and infant with a lateral neck cyst and respiratory distress. There is little dispute that surgery is required for a fourth branchial cyst. If any debate exists, it would be about when to operate. Because of the high incidence of secondary infection, early excision of the cyst is recommended.[20] Identification and complete excision of the entire lesion is essential to the success of treatment.

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CLINICAL NOTE

IRREVERSIBLE SENSORINEURAL HEARING LOSS AS A RESULT OF AZITHROMYCIN OTOTOXICITY

A CASE REPORT

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Azithromycin, an azalide antibiotic, rarely causes ototoxicity. According to the few reports in existence, azithromycin-induced ototoxicity occurred following prolonged high-dose therapy in patients with acquired immunodeficiency syndrome and resulted in a reversible sensorineural hearing loss. We present a case of irreversible sensorineural hearing loss due to azithromycin ototoxicity in an otherwise healthy woman following low-dose exposure to azithromycin.

KEY WORDS — azalide, azithromycin, macrolide, mitochondrial DNA, ototoxicity, sensorineural hearing loss.

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chlear disease was excluded via auditory brain stem response testing, which showed I-V interval latency of <4 ms bilaterally and intertympanic wave V latency of <0.4 ms, and via magnetic resonance imaging.

At subsequent examination 12 months later, the patient still complained of bilateral tinnitus, although it had somewhat improved. Audiometric findings were unchanged compared to those of the initial examination.

DISCUSSION

Macrolide ototoxicity was first described in the early 1970s as a result of erythromycin administration.[3] Of the approximately 30 cases of erythromycin-induced ototoxicity that have been reported in the English-language literature, only 2 have involved irreversible SNHL.[4,5] The ototoxic effects of erythromycin typically consist of a temporary, pan-frequency SNHL and tinnitus and usually occur within the first 72 hours of treatment. Patients with renal or hepatic dysfunction may be at increased risk, as are patients who are treated with doses of 4 mg/d or more.

Of the 3,995 patients who received azithromycin during its phase II and III studies of safety and efficacy, 1.3% of patients complained of headache and/or dizziness and 3 patients complained of "severe dizziness."[6] There were no reported complaints of tinnitus or hearing loss. No audiometric changes occurred in the 62 patients who had standard audiometry performed.

Azithromycin-induced ototoxicity was first described in the treatment of disseminated *M avium* in AIDS patients.[1] These patients received a combination of azithromycin dihydrate (500 mg orally 4 times per day), clofazimine (100 mg orally 4 times per day), and ethambutol hydrochloride (15 mg/kg orally 4 times per day) on a long-term basis. Of the 21 patients undergoing this protocol, 3 patients reported spontaneous hearing loss within 30 to 90 days of initiating treatment. Audiometry confirmed bilateral mild-to-moderate SNHL that resolved 2 to 4 weeks following abatement of azithromycin. One patient was rechallenged with azithromycin when his symptoms returned to normal, and he noticed a hearing loss within 14 days.

In a retrospective analysis of 46 AIDS patients being treated with azithromycin for *M avium* or toxoplasmosis, 8 patients were thought to experience ototoxicity.[2] All 8 received azithromycin (600 mg orally 4 times per day) for a mean of 7.6 weeks. Four patients exhibited mild-to-moderate SNHL, and 1 had an isolated reflex decay alteration. Hearing loss resolved in the 6 patients who had repeat evaluations within a mean of 4.9 weeks.

According to Pfizer, Inc (personal communication, 1998), as of June 1997, there were 10 spontaneous worldwide reports of deafness, 39 reports of decreased hearing, 6 reports of earache, 1 report of hyperacusis, 4 reports of tympanic membrane perforation, and 31 reports of tinnitus. They note that causality of symptoms has not been defined in most of these cases. It was not specified whether the symptoms were of a temporary or permanent nature, or if audiometric documentation of hearing loss was obtained.

Previously published cases of azithromycin ototoxicity describe temporary threshold shifts in pure tone audiometry that resolved within weeks. Our case differs in that it is the first published evidence of a permanent SNHL due to azithromycin. The patient in our report experienced symptoms rapidly after a low-dose exposure to azithromycin, whereas the others developed an ototoxic reaction after a prolonged, high-dose course. In addition, our patient had no confounding medical ailments and was not receiving any other medical therapy.

The earlier reports of azithromycin-induced hearing loss describe an SNHL involving "conversational" frequencies, although the audiometric data were not detailed. Most cases of erythromycin ototoxicity also demonstrate temporary pan-frequency threshold shifts. A similar pattern of reversible, flat SNHL has been described with ischemic changes due to salicylate and quinine ototoxicity. Perhaps these cases of macrolide ototoxicity result from a similar cause. Our patient demonstrated irreversible, high-frequency threshold shifts with hearing that dropped precipitously above 2,000 Hz. This may indicate that the mechanism of injury in our patient differed from that of the others.

Irreversible high-frequency SNHL as a result of ototoxic insult has been evidenced with aminoglycoside antibiotics[7] and chemotherapeutic agents such as cisplatinum.[8] Several mitochondrial DNA mutations have been discovered that result in a propensity for cochlear ototoxicity due to aminoglycosides.[9,10] The mechanism of this SNHL is thought to be related to ion imbalances within the cochlea due to diminished oxidative phosphorylation capacity, resulting in cell damage of the stria vascularis and outer hair cells.[11] By further decoupling oxidative phosphorylation by inhibiting protein synthesis via ribosomal binding, aminoglycosides may accelerate the cochlea damage. Azithromycin also exerts its bactericidal e fect via ribosomal subunit binding. It is possible t the cochlear ototoxicity demonstrated in our pat may result from a similar cause. Furthermore, azithromycin is rapidly distributed into tissues, which results in an intracellular concentration much higher than plasma or serum levels. This increased intracellular concentration may contribute to its potential toxicity.

Finally, it should be noted that azithromycin is not indicated for urinary tract infections other than those due to *Chlamydia trachomatis*. The treating physician did not determine the specific pathogen causing this patient's infection before prescribing azithromycin. This case serves as an example of how possible misuse of an antibiotic can be deleterious to patient welfare.

CONCLUSIONS

We have presented a case of irreversible SNHL occurring as a result of azithromycin ototoxicity. To our knowledge, this is the first published report of permanent hearing loss due to azithromycin. Our patient experienced a rapid onset of symptoms despite a short-term, low-dose exposure to azithromycin. A mechanism similar to that of patients with mitochondrial mutations causing susceptibility to aminoglycosides may explain these findings.

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IMAGING CASE STUDY OF THE MONTH

CHORDOMA OF THE CERVICAL SPINE

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INTRODUCTION

Chordoma is a slow-growing, localized neoplasm arising from the remains of the embryonic notochord. This is a rare tumor, representing at most 5% of all malignant tumors of bone,[1] and demonstrates a large spectrum of biological behavior. Chordoma may be found in several sites: the sacrococcygeal region, the spheno-occipital region, and the mobile spine. The

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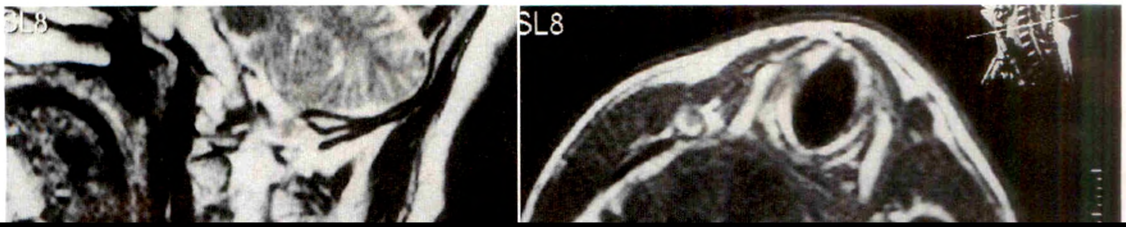
dysphagia and cough had also been noted. The pain prevented normal use of his right hand.

Physical examination revealed a large, firm, immobile mass, anteriorly displacing the larynx. The vocal cord movement was intact. Findings on neurologic examination were normal, except for mild sensory deficit in the first 2 digits of the right hand, in the distribution area of the root of C6.



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The patient underwent resection of the tumor by an anterior cervical approach. A firm, adherent mass was exposed and removed after we dissected it from the exposed ipsilateral vertebral artery. Macroscopically, the cut specimen showed a yellowish white, cystic mass. Microscopic examination revealed large, plump, epithelial-like cells arranged in clusters inside extracellular mucin, compatible with the classic features of chordoma. After the operation, the dysphagia and cough were greatly relieved, the pain was markedly reduced, and the patient regained the ability to use his right hand. Paresthesia of the first 2 digits persisted. Because of involvement of the C6 vertebral body with tumor, the patient was referred for radiotherapy.

DISCUSSION

Between 13.5% and 20% of all chordomas originate in the mobile spine, and the rest originate in the sacrococcygeal and spheno-occipital regions.[1-4] Cervical tumors constitute 28% to 48% of all mobile spine chordomas.[1,3-5] There were only 3 reports of C7 cervical chordomas in 50 cases reviewed.[6]

The presenting symptoms of cervical chordoma are pain, often with a radicular component, and brachialgia. Anterior growth usually leads to dysphagia and other symptoms of a retropharyngeal mass.[2,3,6] Most patients are symptomatic for more than a year before the diagnosis is made.[3,6-8]

Both CT and MRI are useful in demonstrating the extent of the tumor and in postsurgical identification of local recurrences,[7,9] but diagnosis of the tumor can be established only by histopathologic examination. Computed tomography may demonstrate destruction of vertebral bodies, a prevertebral soft tissue mass, and osteosclerosis.[7] The lobular nature of the tumor may be well defined by contrast-enhanced CT scans.[7] This finding, mentioned first by Anegawa et al,[7] is clearly seen in our CT and MRI scans (Figs 1B and 2A,B). Twelve of 14 spinal tumors reported[9] showed destruction limited to 1 vertebra only, as seen in our case.

The preferred treatment is a complete surgical removal of the tumor, which is often very difficult to obtain due to the tumor's high rate of local invasiveness. Although this tumor is relatively radioresistant, adjuvant radiotherapy is recommended.[3-8] Recent reports suggest that high radiation doses, ie, 5,000 to 7,500 cGy, are effective in achieving local control.[4,5] Local recurrence is very common and should be treated surgically and by radiotherapy.

The tendency of chordoma to metastasize is not well established; however, the common sites for metastases are soft tissue, lymph nodes, lung, bone, and liver.[3,8] The incidence of metastases varies from 3% to 60%,[1,3,4,6] with higher incidence incurred in chordomas of the mobile spine than in sacral lesions. Metastases may appear between 1 and 10 years after the initial diagnosis. The presence of metastases does not usually alter the prognosis of the patient, and death is caused in most patients from local recurrence and secondary effects of paraplegia such as infection.[1,3,6]

The 5-year survival rate for treated patients with mobile spine chordoma is 50% to 58%.[1,3,6] The only parameter that seems to influence survival rate is complete resection of the tumor.[6] Variation in histologic pattern does not correlate with biological behavior,[1] and no correlation has been found with age, sex, type of treatment, or tumor site.[3] Further, the indolent growth of the tumor in some patients, in contrast to rapid and massive aggressive recurrences in others, is yet unexplained.[3]

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